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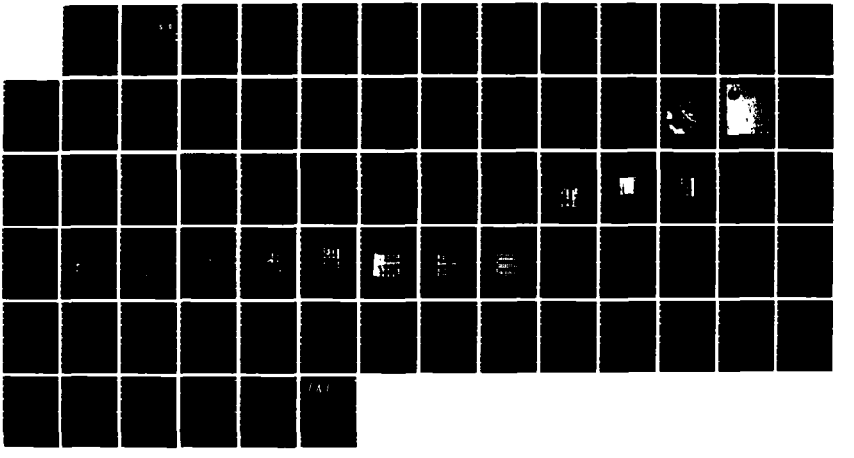

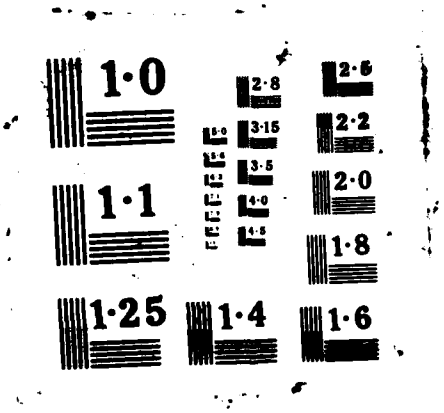
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WORLD REFERENCE CENTER FOR ARBOVIRUSES

ANNUAL PROGRESS REPORT

Robert E. Shope, M.D.

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SUMMARY

Identification and classification of viruses. Viruses were identified in the families Bunyaviridae, Rhabdoviridae, Reoviridae (Orbivirus), Arenaviridae, Flaviviridae, and Togaviridae (Alphavirus). Two new phleboviruses were recognized in Colombia, and new uukuviruses from Australia and North America. Crimean-Congo hemorrhagic fever virus was identified from western China. A new relative of vesicular stomatitis virus was confirmed from Argentina. Orbiviruses continue to be isolated from mosquitoes tested in C6/36 mosquito cells. An orbivirus from Israel is the third recognized member of the new Umatilla serogroup.

Tests at Yale confirmed findings in South Africa that Ippy virus, isolated in 1970 from Mastomys of the Central African Republic, is very closely related to Lassa virus. Electron microscopy revealed rhabdovirus and orbivirus morphology of 2 new mosquito isolates from Indonesia. The rhabdovirus was longer and more slender than other rhabdoviruses so far described. A spherical agent from Peru had a unique morphology with a central dense region and spoke-like projections.

Diagnosis of disease. Sicilian sandfly fever was diagnosed serologically in Swedish troops on Cyprus. This indicates that the virus is still active in the Mediterranean. An alphavirus isolated from Whooping Cranes with fatal encephalitis was confirmed as eastern encephalitis virus.

Serologic and antigen surveys. ELISA survey of human sera of northern Senegal revealed a 6 to 22% Rift Valley fever antibody prevalence including antibody in a 7 year old, indicating relatively recent transmission. The ELISA was also reliable to measure post-vaccination RVF antibody in sheep.

Development of new techniques. RNA-RNA dot blot hybridizaton of orbiviruses represents a new technique for rapid and inexpensive demonstration of the relatedness of each RNA segment of orbiviruses. In a study of reoviruses and Palyam group orbiviruses the test was applicable not only to refined identification, but also to determination of evolutionary patterns and geographic correlations among isolates.

The 4G2 polyvalent flavivirus-specific monoclonal antibody was used in ELISA to capture antigens. This antibody as far as tested was a universal flavivirus capture reagent. It was dried on 96-well ELISA plates and stored at ambient temperature for several weeks without losing efficacy. Polyvalent alphavirus-specific monoclonal antibodies had an extremely wide spectrum among 15 alphaviruses tested by IFA and CF. These should be useful also as grouping reagents.

An ELISA was also developed and applied for serological testing of gulls.

Collection of low passage arbovirus reference strains. Ninety-three additions were made to the collection. These included LaCrosse, Venezuelan encephalitis, dengue, eastern encephalitis, St. Louis encephalitis, yellow fever, western encephalitis, Japanese encephalitis, and vesicular stomatitis viruses from many parts of the world.

Distribution of reagents. The reference center distributed 970 ampoules of reference sera, antigens, and viruses during 1984; mosquito cells and colonized insects were also sent to other laboratories. Of the viruses distributed, there were represented 185 different serotypes.

FOREWARD

In conducting the research described in this report, the investigator adhered to the "Guide for Laboratory Animal Facilities and Care", as promulgated by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences - National Research Council.

I. IDENTIFICATION AND CLASSIFICATION OF VIRUSES

BUNYAVIRIDAE

Viruses from Colombian sand flies (R.B.Tesh). Four viruses, designated CoAr 170187, CoAr 170150, CoAr 170152 and CoAr 170155, were isolated in Vero cell cultures from pools of phlebotomine sand flies collected from human bait during field studies in Colombia in July 1984. Lab Tech slides of Vero cells infected with each of these agents were tested by IFAT against grouping and/or specific antisera prepared against all of the known sand fly-associated arboviruses. Preliminary studies indicate that CoAr 170187, CoAr 170150 and CoAr 170152 (all isolated from mixed species pools of female flies from Arboledas, Norte de Santander) are identical and that CoAr 170155 (isolated from a pool of male Lutzomyia longipalplis collected in Callejon, Tolima) is distinct. Antigenically the four viruses are all members of the phlebotomus fever serogroup. Plaque reduction neutralization tests are planned in the near future to compare these viruses with the 38 known phlebovirus serotypes and to determine if the Colombian isolates represent new or existing serotypes. Laboratory colonies of the principal man-biting sand fly species (Lu. spinocrassa, Lu. gomezi and Lu. longipalpis), present at the two Colombian study sites, have recently been established and their vector competence for the viruses will be tested.

Identification of Aus MI-19334 (A.J. Main). This virus, isolated from Ixodes uriae on Macquarie Island, Australia, was previously reported as a new ungrouped virus of the family Bunyaviridae and named Precarious Point. However, CF tests place it as a new member of the Uukuniemi serogroup (Uukuvirus) (Table 1).

Identification of RML-105355 (A.J.Main). This isolate, recovered from Ixodes uriae in the Northern Hemisphere, was also identified as a member of the Uukuniemi serogroup, similar or identical to murre virus (1983 YARU Annual Report) by CF (Table 1).

Crimean-Congo hemorrhagic fever viruses from China (L. Lee and R.E. Shope). Two strains of virus isolated in 1968 from the Xinjiang Province of western China were referred for identification by Dr. Gao Shou-yi of the Institute of Epidemiology and Microbiology, China National Center for Preventive Medicine, Beijing, China. The strains were isolated from Hyalomma asiaticum and sheep in the epidemic area by a working group of the Chinese Ministry of Health and were linked serologically to the outbreak of Xinjiang hemorrhagic fever of 1966. The tick isolate, HY-68013 was selected for study at Yale. Mice were immunized and a sucrose-acetone extracted brain antigen was used for hemagglutination and for complement-fixation. HY-68013 virus was compared by CF and HI test with the reference Ib Ar 10020 strain of Congo virus and shown to be indistinguishable. The results are presented in Table 2.

Table 1

Complement-fixation tests comparing Precarious Point virus (AusMI-19334) and RML 105355 virus with other members of the Uukuniemi serogroup

	Ascitic Fluid		
	Precarious Point AusMI-19334	Murre Virus	unnamed virus RML 105355
AusMI-19334	512/≥128	256/128	128/64
Murre Virus	64/64	≥512/≥128	≥1024/≥128
RML 105355	64/64	≥512/≥128	≥1024/≥128
FinV-707	32/64	128/64	1024/≥128
UK FT/254	64/8	128/64	64/32
RML-38	32/16	32/16	16/32
LEIV 21c	8/8	8/8	0
S-23	32/32	64/64	256/64
Argas 27	32/32	256/32	256/64
EgAn 1825-61	64/128	128/32	256/64
PakT 462	8/4	32/32	128/16

	unnamed virus FinV-707	unnamed virus UK FT/254	Oceanside Virus RML-38	Zaliv Terpeniya LEIV 21c
AusMI-19334	64/32	16/16	0	16/16
Murre	64/32	16/16	16/16	32/8
RML-105355	16/16	0	0	0
FinV-707	1024/≥128			
UK FT/254		512/32		
RML-38			256/64	
LEIV 21c				32/8

	Uukuniemi virus (S-23)	Grand Arbaud (Argas 27)	unnamed virus (EgAn1825-61)	Manawa virus (PakT462)	Group Uukuniemi
AusMI-19334	64/32	16/16	128/32	0	128/16
Murre	128/8	16/8	256/16	16/4	256/16
RML-105355	32/8	0	64/16	0	64/8
S-23	512/64				
Argas 27		≥1024/≥128			
EgAn 1825-61			≥1024/≥128		
PakT 462				≥1024/≥128	

Table 2

Comparison of Xinjiang hemorrhagic fever virus strain HY-68013 with Crimean-Congo hemorrhagic fever virus strain Ib Ar10200 by complement-fixation and hemagglutination tests

Complement-fixation

	Mouse ascitic fluids	
Antigens	HY-68013 (XHF)	Ib Ar10200 (CCHF)
HY-68013	2048/1024*	2048/1024
Ib Ar10200	2048/1024	4096/1024

*reciprocal of antibody titer/reciprocal of antigen titer

Hemagglutination-inhibition

	Mouse ascitic fluids		
Antigens	HY-68013	Ib Ar10200	Normal
HY-68013 (4 units)	1:1280	1:2560	1:20
Ib Ar10200 (2 units)	1:1280	1:10,240	1:20

RHABDOVIRIDAE

A new Vesiculovirus from Argentina (R.B.Tesh). A mosquito isolate, designated AG83-1347, was sent to YARU by Dr. C. H. Calisher, Centers for Disease Control, Fort Collins, Colorado, for identification. In CF tests performed at the CDC laboratory in Fort Collins, an antigenic relationship was noted between Jurona antiserum and AG83-1347 antigen. Accordingly, indirect fluorescent antibody and plaque reduction neutralization tests were done to confirm this relationship. The results of the tests are shown in Table 3 and indicate that AG83-1347 is a new member of the vesicular stomatitis serogroup (genus Vesiculovirus), related to but distinct from Jurona virus.

Table 3
Relationship of AG83-1347 virus to Jurona virus antibody

Antigen	IFAT		PRNT	
	Jurona	AG83-1347	Jurona	AG83-1347
Jurona	1:1280*	1:10	1:10240	1:10
AG83-1347	1:80	1:20	1:10	1:40

*Highest positive antiserum dilution.

REOVIRIDAE, Orbivirus

Characterization of viruses in mosquito cell cultures (R.B. Tesh, R.E. Shope and D.L. Knudson). During the past three years, we have received about 200 viruses which were originally isolated from mosquito pools in mosquito cell cultures (C6/36 or AP-61). Most of these agents are not pathogenic for newborn mice and do not produce cytopathic effect in mammalian cells on initial passage. Many of them appear to be new viruses (possibly naturally occurring mosquito viruses), since they do not react with the standard arbovirus grouping reagents. A more detailed description of the origin of these viruses was given in the 1983 Annual Report.

Polyacrylamide gel electrophoresis (PAGE) done on these virus isolates indicates that about half of them are 10 segmented RNA viruses. Four mosquito isolates from Indonesia (JKT-7377, JKT-7380, JKT-7398 and JKT-7400) are identical by PAGE with Matsu virus, an orbivirus-like agent first recovered from Culex mosquitoes on Taiwan. By immunofluorescence, a number of the other 10 segmented viruses appear to be members of the bluetongue-epizootic hemorrhagic disease of deer (EHD) serogroup. Two of the mosquito cell isolates in this category (NT-192 and JKT-9133) were selected for further study. NT-192 virus was originally recovered from Culex pipiens mosquitoes by J. Peleg in Israel. JKT-9133 was isolated by J.D. Converse from Anopheles vagus mosquitoes in Indonesia.

Results of complement-fixation and indirect fluorescent antibody (IFA) tests done with JKT-9133, NT-192 and selected orbiviruses are shown in Tables 4

Table 4

Results of CF tests with selected orbiviruses

ANTIGEN	ANTIBODY									
	NT-192	Umatilla	Llano Seco	Bluetongue 10	EHD-NJ	EHD-ALB	IbAr 22619	IbAr 33853	JKT-9133	Tilligerry
NT-192	256*	4	32	0	0	-	0	0	0	0
Umatilla	64	32	64	0	0	-	0	0	0	0
Llano Seco	64	16	64	4	0	-	0	0	0	0
Bluetongue-10	0	0	8	32	0	-	0	4	0	4
EHD-New Jersey	8	0	0	4	16	-	64	128	16	0
EHD-Alberta	8	0	0	8	8	-	64	128	32	4
IbAr 22619	0	0	8	4	8	-	32	128	16	4
IbAr 33853	0	0	4	0	8	-	32	128	16	0
JKT-9133	0	0	8	4	4	-	32	64	64	0
Tilligerry	0	0	4	0	0	-	0	0	0	512

*Reciprocal of highest positive antibody titer. 0 = <1:10.

Table 5

Results of indirect fluorescent antibody tests with selected orbiviruses

ANTIGEN	ANTIBODY									
	NT-192	Umatilla	Llano Seco	Bluetongue 10	EHD-NJ	EHD-ALB	IbAr 22619	IbAr 33853	JKT-9133	Tilligerry
NT-192	320*	40	80	10	10	0	0	0	0	20
Umatilla	160	80	320	0	0	0	10	0	20	20
Llano Seco	80	80	640	0	0	0	0	0	0	20
Bluetongue-10	40	40	10	320	40	10	0	640	-	320
EHD-New Jersey	40	0	10	20	160	160	40	640	80	40
EHD-Alberta	20	0	20	40	40	≥1280	160	≥1280	80	160
IbAr 22619	20	0	20	80	80	NT	160	≥1280	80	20
IbAr 33853	40	≥20	10	80	80	160	80	≥1280	80	80
JKT-9133	20	0	10	20	20	160	80	40	320	80
Tilligerry	10	0	0	10	20	40	20	160	20	≥1280

*Reciprocal of highest positive antibody titer. 0 = <1:10.
NT = Not Tested.

and 5. In these studies, infected Vero cells served as the source of antigen, since NT-192, JKT-9133 and Umatilla viruses do not produce disease in mice. Our results (Tables 4 and 5) indicate, subject to further testing, that NT-192 is a new virus, closely related antigenically to Umatilla and Llano Seco viruses, and that these three agents constitute a complex within the bluetongue - EHD serogroup. Umatilla and Llano Seco viruses were previously classified as ungrouped orbiviruses. JKT-9133 virus appears to be a new member of the EHD complex. Neutralization tests are now in progress to confirm these findings.

Another group of 19 double stranded RNA viruses, isolated from Indonesian mosquitoes collected by J. D. Converse, appears to be in the Orungo virus complex. One member of this group, designated JKT-8132 was selected for further study, since it killed mice after intracerebral inoculation. Results of IFA tests with JKT-8132 and the prototype strain of Orungo virus are shown in Table 6.

Table 6

Immunofluorescence test relationship of Orungo and JKT-8132 viruses

	Antibody	
Antigen	Orungo	JKT-8132
Orungo	1:80	1:40
JKT-8132	1:10	1:80

Experiments were also done to determine the growth of NT-192 virus in mosquitoes. About 100 female Culex pipiens were inoculated with a suspension of NT-192 virus. The mosquitoes were held at 27°C on 10% sucrose; 5 insects were sampled each day. Mosquitoes were triturated individually and each suspension (after centrifugation) was titrated in microplates of C6/36 cell cultures. Plates were examined for evidence of cytopathic effect and the amount of virus was calculated as the C6/36 tissue culture infectious dose$_{50}$ per insect. The results are shown in Table 7. By the second day, titers of the virus had increased significantly; by the seventh day, titers of positive insects were in the range of $10^{8.0}$ - $10^{9.0}$ TCID$_{50}$. Comparable high titers were also obtained in C6/36 cells cultures infected with NT-192 virus. Some of the mosquitoes in this experiment were fed on a mouse five days after inoculation and their eggs were tested. A total of 394 F_1 adults emerging from these eggs were tested for presence of infectivity. All were negative, thus there was no evidence of transovarial transmission of NT-192 virus in Culex pipiens.

The significance and public health importance of these new viruses are unknown. However, they do appear to be fairly prevalent in mosquito populations. As more people use mosquito cell cultures for primary isolation of arboviruses, more of these agents undoubtedly will be found. Because of the ease with which orbiviruses undergo genetic reassortment, it is also possible that some of these 10 segmented genomes serve as potential genetic material for the formation of new viruses. In the case of EHD or bluetongue, for example, this could be of considerable importance.

Table 7

Growth of NT-192 virus in Culex pipiens following intrathoracic inoculation

Day post inoculation	Virus titers in infected mosquitoes*
0	<1.0, <1.0, <1.0, 1.0, 1.5
1	<1.0, <1.0, <1.0, 1.5, 2.5
2	4.0, 5.0, 5.0, 5.5, 6.0
3	5.5, 6.5, 7.0, 7.5, 8.0
4	6.5, 7.5, 8.0, 8.0, 8.5
5	7.5, 7.5, 8.0, 8.0, 8.5
6	<1.0, 8.0, 8.0, 8.0
7	<1.0, 8.0, 8.5, 8.5, 9.0

*Titers expressed as $\log_{10}$ of $TCID_{50}$ per mosquito.

PAGE analysis of recent Mono Lake like virus isolates (J. Oprandy, T. Schwan and A.J. Main). Field samples were collected from birds and ticks in a study sponsored by the Department of Water and Power, City of Los Angeles. Samples that had tested positively for Mono Lake virus by ELISA were analysed by polyacrylamide gel electrophoresis (PAGE). BHK-21 cells were infected and nucleic acids labeled in vivo with P-32. Viral dsRNA was purified as described by Knudson (see previous Annual Reports). Purified viral dsRNA was then electrophoresed, along with other Chenuda complex viruses and Mono Lake prototypes as listed in Table 8, in 10% Laemmli gels. These gels were dried and autoradiographed.

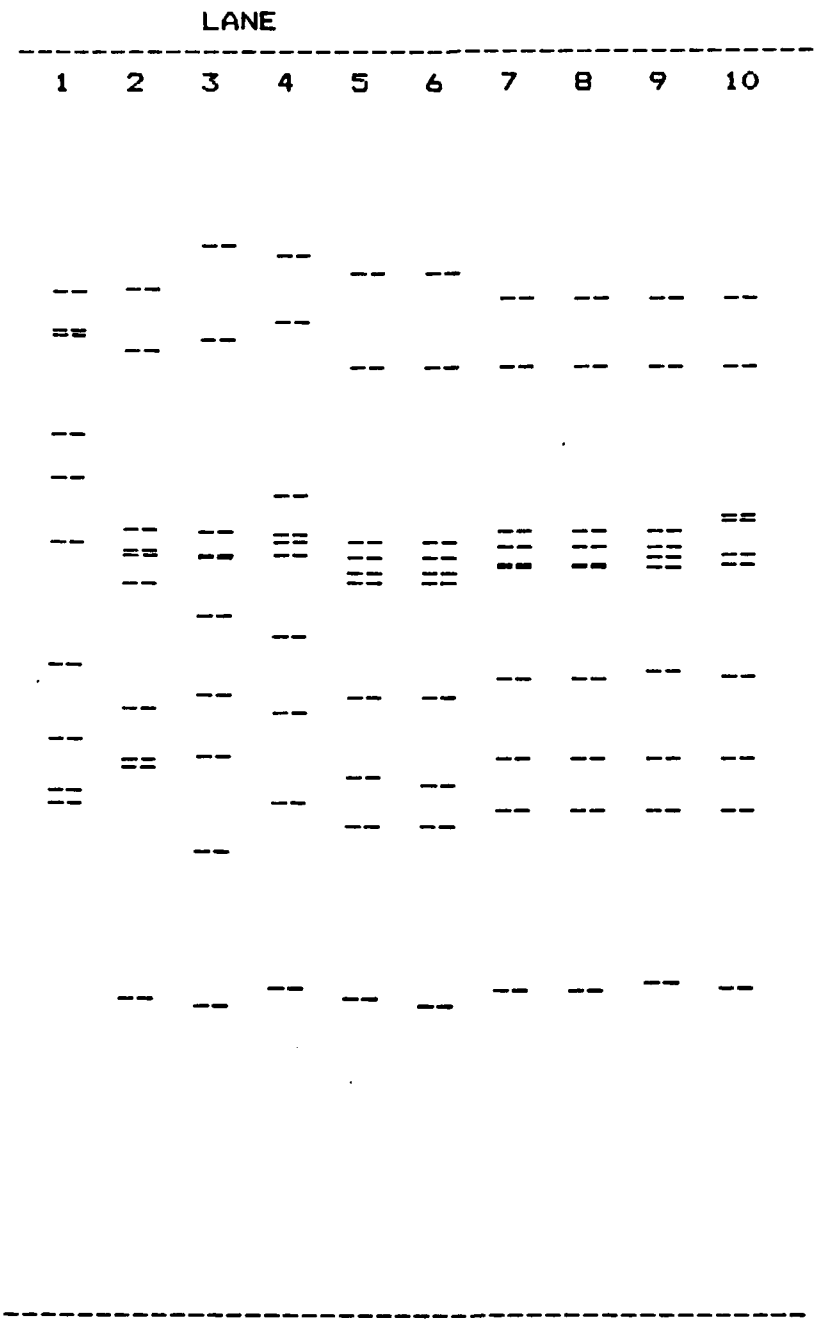
PAGE profiles (Figure 1) of several Mono Lake isolates showed a similar distribution of 10 genomic segments. This distribution differs significantly from those of Huacho, Sixgun City and Chenuda prototype profiles. Mono Lake 66 (CalAr 861) and Mono Lake 82 (CalAr 1614) differ in segments 8 and 10 where the ML 66 gene segments have a slightly higher molecular weight. There have been three genotypes in the 1984 ML isolates observed. Most gene segments of the recent isolates have a similar pattern but are of higher molecular weight than those of the 1966 and 1982 ML isolates. Isolates ML 23 and ML 26 (from Little Norway Island) are identical. Segments 1,2,8 and 9 of the 1984 ML-like isolates comigrate. In addition, segment 4 of ML 23, ML 26 and ML 32 comigrate. Segment 2 comigrates in all Mono Lake isolates tested.

Table 8

Chenuda complex viruses examined by PAGE

Lane	Virus	Strain	Location	Year
1	Reovirus-3	MVM lung 66-23	California	1968
2	Huacho	Cal Ar 883	Peru	1966
3	Sixgun City	USA RML 52451	Texas	1969
4	Chenuda	Eg Ar 1152	Egypt	1954
5	Mono Lake	Cal Ar 861	Mono Lake, USA	1966
6	Mono Lake	Cal Ar 1416	Mono Lake, USA	1982
7	Mono Lake	ML23	Little Norway Island	1984
8	Mono Lake	ML26	Little Norway Island	1984
9	Mono Lake	ML32	MacPherson-D Island	1984
10	Mono Lake	ML33	MacPherson-D Island	1984

Figure 1. Graphic representation of PAGE of Chenuda Complex viruses. Identification for lanes is in Table 8.



ARENAVIRIDAE

Ippy virus relationship to Lassa (R. Cedeno, G.H. Tignor, R.E. Shope). Notification was received from Dr. R. Swanepoel, National Institute for Virology, Sandringham, South Africa that he had found a cross-reaction by IFA between Ippy virus and serum of Lassa virus. At Yale, a mouse immune serum prepared in 1979 by Dr. J. Casals was tested by IFA with spot slides of Lassa virus supplied by Dr. J. McCormick, CDC, Atlanta. The Lassa-infected cells reacted strongly and unambiguously with the Ippy serum. The Yale stocks of the virus were shipped to CDC, Atlanta and to USAMRIID, Fort Detrick where further work with Ippy virus can be pursued under P4 biocontainment.

FLAVIVIRIDAE

Virus isolates from Viet Nam (R.B.Tesh and R.E. Shope). Seven lyophilized mouse brain suspensions were received from the National Institute of Hygiene and Epidemiology, Viet Nam via Dr. Judith Ladinsky, University of Wisconsin, for virus isolation and identification. These specimens had been tentatively identified in Viet Nam as containing Japanese encephalitis (JE) or dengue viruses. Brain suspensions were reconstituted and inoculated into cultures of C6/36 cells. After 7 days of incubation, spot slides of the infected mosquito cells were prepared and examined by indirect fluorescent antibody test against the following reagents: dengue types 1,2,3 and 4 specific monoclonal antibodies as well as chikungunya and JE polyclonal mouse immune ascitic fluids. In IFAT, 6 of the specimens were positive with the JE antibody; the seventh failed to react with any of the reagents used.

The isolates were subsequently inoculated into baby mice. Five of the 7 isolates killed mice. Brains of these and of 2 similar isolates sent to Yale last year by the same laboratory, reacted by CF test to homologous titer with JE reference immune ascitic fluid and to only a low titer with ascitic fluids of dengue types 1 and 3.

Identification of NCAr 323 from New Caledonia (W.K. Wu, R. Tesh, A.J. Main): This strain, isolated from a pool of Culex bitaeniorynchus in New Caledonia and referred by the Institut Pasteur, was placed in group B by CF and HI (Table 9) and identified as dengue 4 by ELISA and IFA using monoclonal antibodies (Table 10).

Table 9

Comparison of NC Ar 323 with selected group B viruses by complement-fixation and hemagglutination-inhibition.

	NC AR 323 ANTIGEN		NC AR 323 ANTIBODY	
	CF	HI	CF	HI
DENGUE 1	256/256		512/512	
DENGUE 2	8/16		256/512	
DENGUE 3	8/16		256/512	
DENGUE 4	1024/1024		1024/512	
ALFUY	-		64/512	
EDGE HILL	16/64		32/512	
STRATFORD	8/32	<10/-	128/512	
WESSELSBRON	8/32		8/512	
KUNJIN		80/5120		≥5120/2560
MURRAY VALLEY ENCEPH.		40/320		1280/2560
KOKOBERA		-		80/2560
SEPIK		5120/-		320/2560
TEMBUSU		10/-		

Table 10

Comparison of NC Ar 323 by ELISA and IFA

monoclonal antibodies

	DEN-1	DEN-2	DEN-3	DEN-4	Pooled
NC Ar 323	+ (-/-)*	- (-/-)	- (-/-)	+ (+/+)	+
DEN-1	-	-	-	-	+
DEN-2	- (-/-)	+ (+/+)	- (-/-)	- (-/-)	+
DEN-3	-	-	-	-	+
DEN-4	+	-	-	+	+
NORMAL	- (-/-)	- (-/-)	- (-/-)	- (-/-)	-

* ELISA results; IFA results in parentheses (antigen 1/antigen 2)

TOGAVIRIDAE Alphavirus

Identification of contaminants in virus stocks. (A.J.Main). Dr. Charles Calisher, CDC, Fort Collins, and Dr. Thompson from the National Institute of Virology, Sandringham, South Africa reported an Alphavirus in stocks prepared at Yale of three ungrouped viruses from Africa - Landjia (DakAnB 769d), Zingilamo (DakAnB 1245a), and Kolongo (DakAnB 1094d). The Alphavirus is presumably a contaminant. CF tests at YARU, done with new ascitic fluids made from Landjia and Zingilamo stocks showed cross reactions with Aura, Sindbis, Whataroa, WEE, Fort Morgan, Highlands J, Semliki Forest, Ross River, Getah, VEE, Mayaro, and Boteke; fresh antigens crossed with WEE, Mayaro, Getah. They also reacted with Boteke and Kolongo. Drs. Calisher and Thompson later identified the contaminant as Semliki Forest virus.

Identification of isolates from Connecticut (A.J. Main). Virus was isolated from eight pools of Culiseta melanura collected during 1984 as part of a field study on Lyme disease and other zoonoses in Connecticut. A ninth strain was recovered from a pool of Culex restuans. Five of these strains, including the one from Cx. restuans, were identified as Flanders virus by CF, two as Highlands J and two as EEE.

MORPHOLOGICAL IDENTIFICATION OF VIRUSES

Electron microscopic studies of mosquito isolates (T.G. Burrage, R.B. Tesh, J.G. Olson and L. Walters). Electron microscopic studies were carried out on three mosquito isolates obtained from Indonesia and Peru. Strains JKT-6841 and JKT-8126 were provided by Dr. J. Converse, NAMRU-2, Jakarta; and PE 2617-77 by Dr. Mendes of the Universidad Nacional Mayor de San Marcos, Lima, Peru. The mosquito cell line C6/36 was inoculated with each strain and the cells were incubated at 37°C. After 36 h, the infected cells all exhibited 2-3+ CPE (cell rounding, detachment and extensive vacuole formation). The cells ere fixed at this time with a solution containing 3% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4). The cells were postfixed with 2% osmium tetroxide, stained en bloc with uranyl acetate, and embedded in Epon/araldite.

Each strain was tested against reference grouping fluids. No cross reaction was seen with these isolates.

Two strains had unique morphologies. JKT-6844 (Figure 2) was similiar to rhabdoviruses in that it consisted of a slender rod projecting from the plasma membrane. The rod was approximately 50 nm in diameter but its length was extremely variable. No free particles were observed to obtain a definite length. The budding was confined to the plasma membrane and no accumulations of matrix proteins were present. The rod-shaped projection was clearly striated but the surface was not covered with spikes.

Strain 2617-77 (Figure 3) was a spherical agent. The spheres, 60-80 nm in diameter, were located in large vacuoles in the cytoplasm. A smooth-surface endoplasmic reticulum was always associated with the vacuole and the reticulum contained amorphous material. The agent appeared to have a membrane envelope which may have been derived from the wall of the vacuole as several budding profiles were observed. The agent appeared to have a centrally located electron-dense region with spoke-like projections radiating from the center.

The third strain JKT-8126 (Figure 4) had the appearance of an orbivirus. The agent was 50-60 nm in diameter and located in the cytoplasm and in the vacuoles. Accumulations of viral proteins, electron-dense amorphous masses, were present also in the cytoplasm. Smooth-surfaced reticula occurred in the cytoplasm and contained viral particles in various stages of maturation. The mature particle was enveloped and had an electron-dense core.

Figure 2. A rhabdovirus-like particle (strain JKT-6844). Four profiles are present in this section. The particle is emerging from the plasma membrane. The bar is 70 nm.

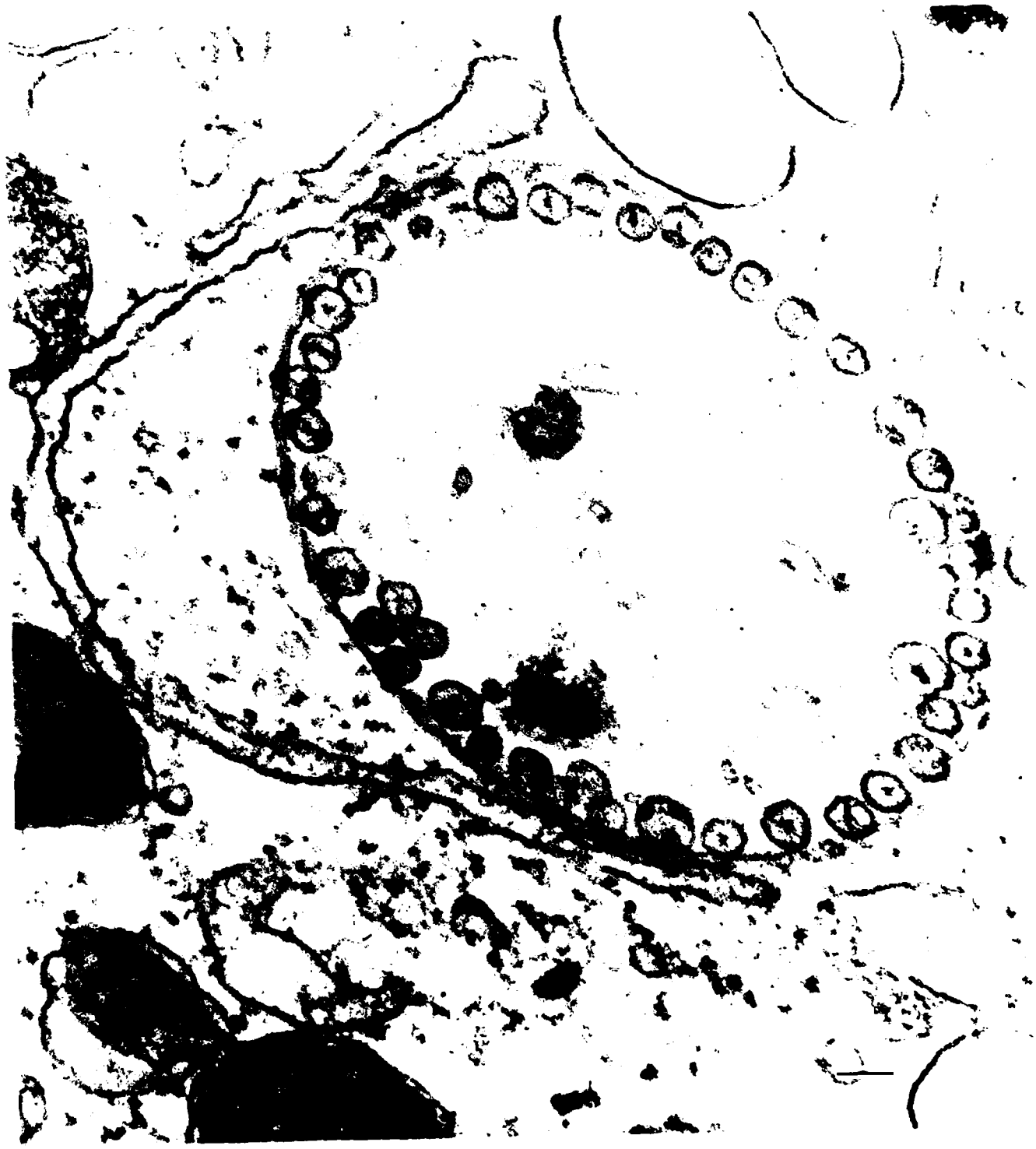


Figure 3. A spherical agent (Strain 2617-77) present in a vacuole in C636 cells. The bar is 70 nm.

Figure 4. An orbivirus-like virion in the cytoplasm of C636 cells. The bar is 70 nm.

II. DIAGNOSIS OF DISEASE

Sandfly fever in Swedish soldiers (R.B.Tesh and B. Niklasson). Acute and convalescent sera were obtained from seven Swedish soldiers serving in the United Nations Forces in Cyprus. All of these men had acute febrile illnesses compatible with sand fly (phlebotomus) fever during the summer of 1984. The serum specimens were initially tested by Dr. Bo Niklasson, The National Bacteriological Laboratory, Stockholm, by indirect fluorescent antibody test, using polyvalent spot slides prepared with Naples and Sicilian antigens. Several of the convalescent sera reacted positively by immunofluorescence and the samples were submitted to YARU for confirmation.

All sera were tested by plaque reduction neutralization method against three phleboviruses (Naples, Sicilian and PaAr 814) known to occur in the Mediterranean. Results are shown in Table 11. Patients 1, 3 and 4 showed a serologic conversion to Sicilian sand fly fever virus, and patients 2,5 and 6 had Sicilian neutralizing antibodies in their convalescent sera. Acute phase sera were not available on these latter men. Patient 7 did not develop antibodies to any of the viruses tested. These results indicate that six of the men were probably infected with Sicilian sand fly fever virus. The data also demonstrate that phlebotomus fever is still present in the Mediterranean region. Historically, this disease has been a military problem; during World War II, it was a major health problem for British, American and German troops in Italy, Greece, Palestine and North Africa. Virus activity occurs annually in endemic areas and becomes apparent when non-immune persons (i.e. soldiers and tourists) enter the region.

Equine and avian encephalitis in Connecticut, 1984 (A.J. Main). Brain samples from two horses and two pheasants, diagnosed clinically and histologically as having died of viral encephalitis, were sent to YARU by Ms. Lois Heinz at the State Diagnostic Service, Department of Pathobiology, University of Connecticut for isolation attempts. EEE virus was recovered from one of the horses and both pheasants.

Single acute blood samples from two sick horses were also received from the Diagnostic Service. One was negative for EEE and Highlands J antibodies by CF and HI; the second was negative by HI, but had a CF titer of 1:16 with EEE antigens. Convalescent serum samples were not available and a diagnosis could not be made in either case.

Virus was not recovered from brain samples from 1 sick and 1 dead penned bobwhite quail. Clinically and epidemiologically, the quail outbreak appeared to have been caused by a bacterial agent.

EEE in Whooping Cranes in Maryland (A.J. Main and T.G.Schwan). Two strains of virus were received from Douglas Docherty of the National Wildlife Health Laboratory in Madison, Wisconsin for identification. These strains were isolated from pooled organs from two of seven Whooping Cranes (Grus americana) that died of an unknown cause at the Patuxent Wildlife Research Center in Maryland during 1984. WiscAn 5000-001 was isolated from the liver, spleen, kidney, and "body fluids" of a male crane which died on September 24, 1984; WiscAn 5000-003 was from liver, spleen, kidney, intestine, and skeletal muscle of a female that died two days later. Both birds were necropsied at the National Zoon in Washington on the day of death. Both strains were identified as EEE virus by ELISA, CF, and HI (Tables 12 and 13).

Table 11

Results of plaque reduction neutralization tests on Swedish soldiers in Cyprus

			Virus		
	Serum number		Sicilian	Naples	PaAr 814
Patient 1	acute	58-841124A	0*	0	0
	conval.	59-841124C	160	0	0
Patient 2	conval.	60-841124C	80	0	0
Patient 3	acute	63-841124A	0	0	0
	conval.	64-841124C	80	0	0
Patient 4	acute	71-841124A	0	0	0
	conval.	72-841124C	160	0	0
Patient 5	conval.	73-841124C	160	0	0
Patient 6	conval.	74-841124C	20	0	0
Patient 7	acute	77-841124A	0	0	0
	conval.	78-841124C	0	0	0

*Reciprocal of highest serum dilution producing >80% plaque inhibition.
0 = <1:10.

Serum XB10620 was also negative against the 3 viruses.

Table 12

Identification of virus isolations from whooping cranes (Grus americana) by complement-fixation.

Antigens	Ascitic Fluids 5000-001	5000-003	EEE	HJ	NORMAL
5000-001	≥1024/≥128	≥1024/≥128	512/≥128	0	0
5000-003	≥1024/≥128	≥1024/≥128	512/≥128	0	0
EEE	512/16	1024/32	1024/16	0	0
HJ	0	0	0	32/4	0
NORMAL	0	0	0	0	0

Reciprocal of serum titer/reciprocal of antigen titer (0 = <8/<4)

Table 13

Identification of virus isolates from whooping cranes (Grus americana) by hemagglutination-inhibition

Antigens	Ascitic Fluids 5000-001	5000-003	EEE	HJ(111)	HJ(8)
5000-001	2560	5120	2560	0	10
5000-003	5120	10240	2560	0	10
EEE	640	2560	2560	10	0
HJ	80	320	80	160	2560

Reciprocal of serum titer (0 = <10)

III. SEROLOGIC AND ANTIGEN SURVEYS

Hantavirus antigen and antibody in voles from Connecticut (A.J. Main and P-W. Lee). Lung samples from 10 meadow voles (Microtus pennsylvanicus) collected in Connecticut were sent to Dr. Pyung-Wu Lee at NIH to examine for Prospect Hill (PH) virus of the genus Hantavirus. Antigen was detected by FAT in one sample. PH antibody was demonstrated in eight of 100 serum samples from meadow voles sent to Dr. Lee.

Use of ELISA to survey for antibody to Rift Valley fever and other phlebovirus antibodies in human sera from northern Senegal (Shirley Tirrell and R.E. Shope). Sera were collected in 1976 from 5 villages of the Senegal River Valley during a US AID-sponsored survey of the arid region of northern Senegal. Testing by immunofluorescence at that time indicated the presence of a relatively high prevalence of antibody to Rift Valley fever (RVF) virus. The positive reactions were confirmed by plaque reduction neutralization test done in the laboratory of Dr. C.J. Peters at USAMRIID, Fort Detrick, Frederick, Maryland.

This present study was initiated to: 1) determine the prevalence rates by village of residence, sex, and age; 2) demonstrate that the ELISA could be used for serosurvey and to investigate its specificity; 3) determine if secondary infection patterns could be detected and whether or not other phlebovirus antibody was confounding the interpretation of positive results.

The RVF ELISA employed pooled monoclonal antibody as coating to capture a mouse liver antigen supplied by the US Army. SFF Sicilian, SFF Naples, Salehabad, Karimabad, Saint-Floris, Gabek Forest, and Gordil phleboviruses were used to detect cross-reacting antibodies. In each case, mouse (or rabbit) sera were ammonium sulfate precipitated and dialyzed, then used as coating antibody with mouse brain antigen. Plates were coated and the antigen added at the beginning of the project, then the antigen-containing plates were stored at -70°C until the day of the test. On the day of the test, human sera were added, followed by anti-human IgG peroxidase conjugate and ABTS substrate with one hour incubation periods between steps. The optical density was determined on a Titertek MicroELISA reader at 414 lambda.

Two hundred forty-nine sera were tested. Data for age and sex were not available for 2 RVF-negative sera, and these sera were not analyzed further. There were positive sera from Lampsar (15/94, 16%), Savoigne (2/18, 11%), Kossack-Sud (1/16, 6%), and Kossack-Nord (15/116, 22%). Three sera from Ronkh were negative. Each village where a large enough sample was collected was positive, and the differences in prevalence may represent sampling bias.

Sixteen % of males and 19% of females were positive. The youngest RVF positive resident was a 7 year old female from Lampsar. The prevalence pattern revealed 4-8% positive reactions in persons under 20 years, 24% in persons 20-39, and 39-42% in persons between 40 and 59 (Table 14). This pattern could be explained by outbreaks at about 20

year intervals, or possibly by endemicity with less exposure of children than adults.

Most of the positive sera reacted only with RVF antigen, or with RVF antigen plus a single other phlebovirus, usually in lower titer. These appear to be primary reactions. A few sera reacted with multiple phleboviruses, usually to a relatively low titer. These are probably secondary reactions, indicating that one or more other phlebovirus has infected the population of the Senegal River Basin. One of these may be Sicilian sandfly fever virus for which there was at least one primary type reaction (Table 15). It appears practical to use ELISA for phlebovirus serosurveys, especially if multiple phlebovirus antigens are employed.

Rift Valley fever ELISA to measure antibody response to vaccination of sheep (J.M. Meegan, R.E. Shope, B.A. Peleg and J. Shay). In a reference center project sponsored in part by the U.S.-Israel Binational Agricultural Research and Development Fund, the ELISA was developed to detect antibody in sheep and cattle. The technique was taken to the Kimron Veterinary Institute where sera of RVF-vaccinated sheep and cattle were available. Many of these sera had been tested previously by HI at the Kimron Institute and by plaque reduction neutralization by Dr. C.J. Peters, Fort Detrick. The correlation of the ELISA, HI, and PRN tests was excellent. Tables 16 and 17 show the ELISA results of sera of sheep inoculated subcutaneously with oil adjuvant inactivated vaccine and with alum adjuvant inactivated vaccine.

With the oil adjuvant vaccine, the ELISA was approximately as sensitive as the HI and PRN tests. Control animals (not shown) were negative in all three tests. Sheep vaccinated with the alum adjuvant vaccine showed minimal responses by HI and PRN tests, yet reacted consistently by ELISA. Again, non-vaccinated sheep were negative. The reason for the increased relative sensitivity of the ELISA over the HI and PRN tests in the alum adjuvant group is not clear. It is possible, however, that the explanation lies in the fact that the ELISA measures both nucleocapsid-reactive antibody and glycoprotein-reactive antibody, while the HI and PRN measure only the glycoprotein-reactive antibody.

Table 14

Rift Valley fever ELISA antibody in the human population of 5 villages of northern Senegal

Age in years	Males	Females	Totals	% positive
0-9	1/23*	1/27	2/50	4
10-19	2/41	4/36	6/77	8
20-29	0/9	11/36	11/45	24
30-39	1/5	5/20	6/25	24
40-49	8/16	3/12	11/28	39
50-59	4/9	1/3	5/12	42
>59	1/6	1/4	2/10	20

*Number of sera with titers >1:50/number tested

Table 15

Patterns of ELISA reactions of human sera from northern Senegal

	Phlebovirus ELISA antigens								
Serum no.	RVF	SSF	SAL	Naples	KAR	St.FL	Gab.F	GOR	Infection pattern
93	1600	0*	0	0	0	0	0	0	primary RVF
184	1600	0	0	0	0	0	0	0	primary RVF
930	400	0	0	0	0	0	0	0	primary RVF
379	6400	0	0	0	0	0	0	0	primary RVF
96	1600	0	0	0	400	0	0	0	? primary
883	3200	200	0	0	0	0	0	0	? primary
808	100	100	0	0	0	≥100	≥100	0	secondary
897	400	200	400	0	0	0	≥100	≥100	secondary
844	0	400	0	0	0	0	0	0	primary SSF

*0 = ≤50

Table 16

Serological response of sheep inoculated subcutaneously with oil adjuvant-inactivated RVF vaccine

Day after vaccination	HI	PRNT80	ELISA
Sheep #332			
14	20480	1280	6400
28	40960	2560	102400
42	655360	10240	102400
56	327630	10240	>102400
84	327630	10240	25600
175	163840	2560	6400
205	163840	2560	6400
Sheep #434			
14	1310000	640	6400
28	655360	1280	25600
42	20480	640	25600
56	20480	1280	25600
84	5120	1280	6400
175	5120	1280	6400
205	5120	1280	6400
416	2560	640	6400

Table 17

Serological response of sheep inoculated subcutaneously with alum adjuvant inactivated-RVF South African commercial vaccine

Day after vaccination	HI	PRNT80	ELISA
Sheep #323			
14	640	40	1600
28	320	40	1600
42	160	40	1600
56	80	20	400
84	80	80	1600
175	160	40	1600
Sheep #369			
14	20	<10	400
28	<10	<10	400
42	<10	<10	400
56	<10	20	400
175	<10	10	100

IV. DEVELOPMENT OF NEW TECHNIQUES

RNA-RNA BLOT HYBRIDIZATION

Assessment of Sequence Relatedness of dsRNA Genes by RNA-RNA Blot Hybridization (D. K. Bodkin and D. L. Knudson) Three well-characterized reovirus serotypes were used to investigate the usefulness of RNA-RNA blot hybridization as a means to assess the genetic relatedness of dsRNA viruses. [5'-^{32}P]-pCp labeled genomic dsRNAs from reovirus 1, 2, and 3 were used as probes in hybridization experiments in which segments of the three serotypes were separated in 10% polyacrylamide gels and were transferred electrophoretically to membranes. Nine of the ten reovirus genes cross-hybridized between the serotypes. The S1 gene was serotype specific. The L2 gene of reovirus 2 showed a lower level of cross-hybridization with types 1 and 3 when compared to the hybridization signal observed for L2 when types 1 and 3 were hybridized to each other. The data were consistent with previous studies on the relatedness of the three virus serotypes. Since RNA-RNA blot hybridization allows the number and identity of conserved genes to be determined, this approach may prove useful for assessing the genetic relatedness among other viruses in the family Reoviridae.

Labeled dsRNA from reovirus 1, 2, or 3 was electrophoresed next to unlabeled lanes of the viruses to verify that dsRNA segments were transferred evenly and to serve as markers for the unlabeled lanes (Figures 5 - 7, lanes A, C, and E). Minor differences were observed in the efficiency of transfer of dsRNA segments. For example, the L1-3 segments appeared to be transferred less efficiently than the other segments (Figures 5 - 7, lanes A, C, and E). Genomic dsRNAs from reovirus 1 (Figure 5), reovirus 2 (Figure 6), and reovirus 3 (Figure 7) were used as probes in three hybridization experiments. The reovirus 1 probe was removed from the filter-bound RNA (Figure 5) and the filter was rehybridized to [^{32}P]pCp labeled dsRNA from reovirus 3 (Figure 7). Comparison of labeled and unlabeled lanes of each serotype (lanes A and B, C and D, and E and F in Figures 5 - 7) revealed segments in the unlabeled lanes which did not hybridize with the probe. In all three experiments performed at 52°C, 50% formamide, 5X SSC, the S1 gene of the probe did not hybridize to the S1 gene of the other two serotypes. The remaining nine genes of the three serotypes exhibited positive hybridization signals. When reoviruses 1 and 3 were used as probes (Figures 5 and 7), the hybridization signal seen with the L2 gene of reovirus 2 was faint compared to the hybridization signals of the other eight reovirus 2 genes. The L2 genes of reoviruses 1 and 3 exhibited levels of cross-hybridization which were equivalent to the other eight genes.

The effect of a reduction in the stringency of hybridization was examined by a hybridization experiment which was performed at 42°C, 50% formamide, 5X SSC using reovirus 3 as the probe. The S1 gene of reovirus 3 did not hybridize to the S1 genes of the other two serotypes at 42°C. The other nine genes cross-hybridized as would be expected based upon the hybridization experiments performed at 52°C (data not shown).

A single gene in the probe may hybridize with several segments in

both homologous and heterologous samples. However, those genes to which several labeled genes hybridize would show stronger signals relative to the other genes in that sample. The probe appears to bind equally to both homologous and heterologous unlabeled genomes for all segments with the exception of the L2 gene of reovirus 2. These data may indicate that each segment in a serotype hybridizes to no more than one segment in another serotype. Moreover, the S2 gene of reovirus 3 was isolated and used as a probe against the three reovirus serotypes. The single S2 gene of reovirus 3 hybridized only with its single cognate gene in each of the three viruses (data not shown). The S1 gene was also similarly prepared, and it hybridized only with its cognate gene in reovirus 3 confirming S1 as the unique gene in these studies (data not shown).

Zeta-probe membrane offers several advantages over nitrocellulose and DBM paper for filter hybridizations. The membrane requires minimal treatment in contrast to DBM paper. Probes can be removed from filter-bound RNA, and the membranes have been rehybridized up to six times without loss of sensitivity. Since end-labeling of genomic dsRNA with [^{32}P]pCp yields evenly labeled segments, differences in intensities of hybridization signals can be correlated with differences in sequence homology between genes. Further, the addition of [5'-^{32}P]-pCp to the 3' ends of genomic dsRNA is straight-forward with few technical steps when compared with incorporation of isotope by mRNA <u>in vitro</u> synthesis from viral cores, or by cDNA preparation from genome segments by reverse transcription. If cDNA clones are available, then they could be used as probes. However, cloning the ten genes of a large number of viruses for the purpose of hybridization analyses is not practical. Since the PAGE dsRNA profile may be diagnostic for a given isolate, the transfer of dsRNA segments from polyacrylamide gels results in an image of the PAGE dsRNA profile on the membrane which ensures that the correct isolate has been used. Likewise, this RNA-RNA blot hybridization approach permits the examination of large numbers of viral isolates.

Reovirus strains which are known to be related by immunological criteria, hybridization in solution, and sequence analysis exhibit a high degree of relatedness by RNA-RNA blot hybridization. The data presented here demonstrate that RNA-RNA blot hybridization may be useful to determine the relative levels of sequence conservation of individual genes and to identify cognate genes between viral isolates. In this laboratory, RNA-RNA blot hybridization is being used to examine the genetic relatedness of members of the 12 serogroups of the <u>Orbivirus</u> genus, to classify ungrouped orbiviruses, and to identify new isolates. Type-specific and conserved genes within the Palyam serogroup of the genus <u>Orbivirus</u> have been identified. Studies of other genera within the family Reoviridae may add to our understanding of the evolution and taxonomic relationships of this large family of dsRNA viruses.

These data are currently in press in the Journal of Virological Methods.

Figure 5. Autoradiogram of a membrane probed with [5'-^{32}P]-pCp labeled dsRNA from reovirus type 1. The lanes are marked uninfected cellular control (U), pCp labeled (A) and unlabeled (B) reovirus 1, pCp labeled (C) and unlabeled (D) reovirus 2, pCp labeled (E) and unlabeled (F) reovirus 3. In each serotype, the three largest genes (top of the figure) are L1-L3, the middle genes are M1-M3, and the smallest genes are S1-S4. The dsRNA PAGE profiles for the three serotypes have been described and the cognate genes between the three viruses have been correlated elsewhere previously. Comparison of labeled and unlabeled lanes of the same serotype allows identification of genes in the unlabeled lanes which did not hybridize with the indicated probe. Samples were electrophoresed at 20 mamps for 20 h through Tris-glycine buffered 10% polyacrylamide gels and transferred to Zeta-probe membrane (Bio-Rad) in 1X TAE at 0.6 amps for 30 min, and then 1.6 amps for 4 h using a Trans-Blot cell (Bio-Rad).

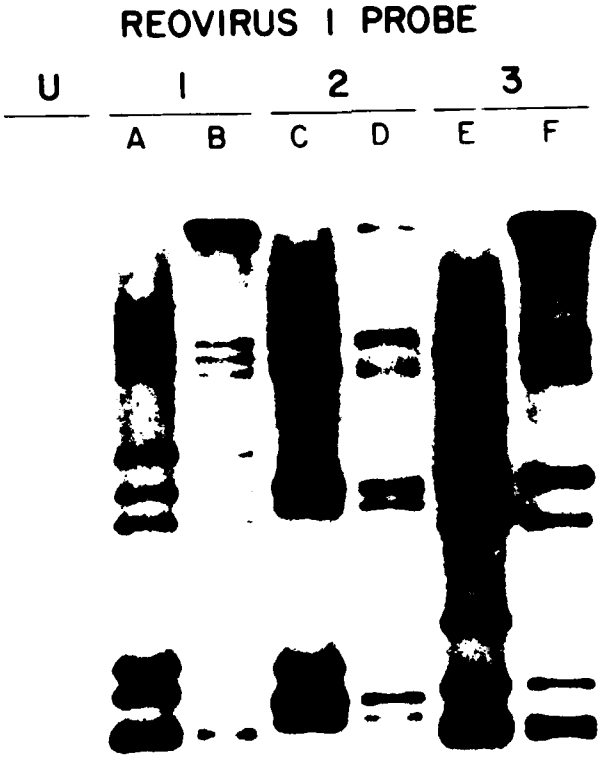


Figure 6 . Autoradiogram of a membrane probed with labeled dsRNA from reovirus type 2 as described (Figure 5).

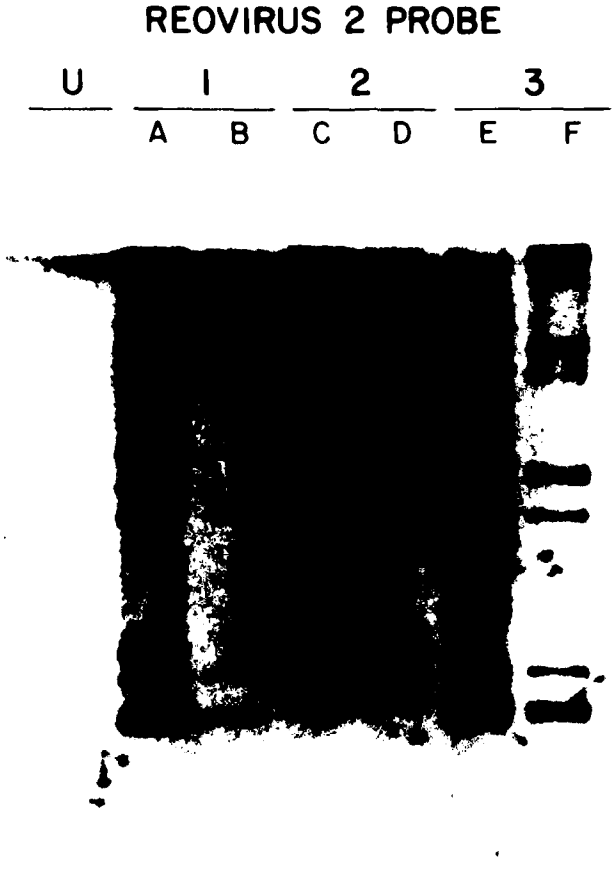
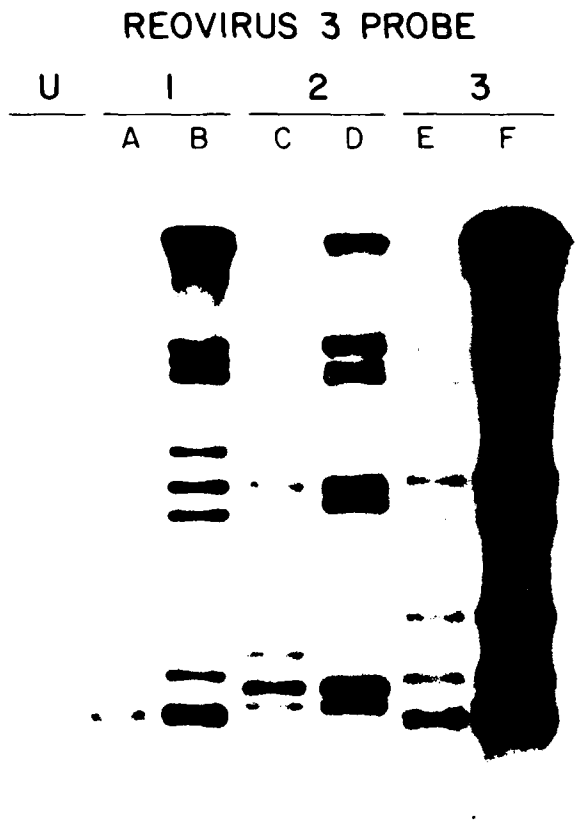


Figure 7. The membrane used in Figure 5 was stripped of its reovirus 1 probe as described in the text and rehybridized using reovirus type 3 as the probe as described (Figure 5).



Sequence Relatedness of Palyam Virus Genes to Cognates of the Palyam Serogroup Viruses by RNA-RNA Blot Hybridization (D. K. Bodkin and D. L. Knudson) Cognate genes of nine members of the Palyam serogroup of orbiviruses have been identified and their relatedness to the prototype, Palyam virus, has been determined. Viral dsRNA segments were electrophoresed through 10% polyacrylamide gels, transferred to membranes, and hybridized to labeled RNA from Palyam virus under hybridization conditions using 52 C, 50% formamide, 5X SSC. Cognate genes of each virus isolate were identified by hybridizing their genomes to [5'-^{32}P]-pCp labeled, isolated segments from Palyam virus. Single segments from Palyam hybridized to no more than one segment in the other isolates. Nine of the ten genes exhibited nucleic acid sequence homology between Palyam and seven of the other eight isolates. Gene 2 of Palyam hybridized only with gene 2 of CSIRO Village, and it was correlated with serotype-specificity. Since CSIRO Village is the only member of the serogroup which cross-reacts with Palyam in neutralization tests, gene 2 may encode the neutralization antigen. Variation in the intensity of the hybridization signals of the remaining nine genes within a given virus indicated that the number and identity of conserved genes differed between members of the group. Genes 5, 7, and 9 were the most conserved genes for all members of the serogroup, while the levels of relatedness of Palyam genes 1, 3, 4, 8, and 10 to their cognates in the other isolates varied under these hybridization conditions.

Serotype-Specific Gene by Dot-Blot Hybridization. The initial identification of the gene which correlated with serotype-specificity of the Palyam serogroup viruses was made using a dot-blot technique. Infected cell-extracted dsRNA from nine Palyam serogroup viruses was spotted onto 10 replicate nitrocellulose filters. Probes consisted of individual Palyam segments isolated from low melting point agarose (Figure 8). Segment 2 was the only gene that did not hybridize to the RNA of the other viral isolates, suggesting that it may be serotype-specific. Comparison of the other nine blots showed roughly equivalent levels of hybridization for each segment (data not shown).

Identification of Cognate Genes. Palyam segments were purified using low melting point agarose and electrophoresed in 10% polyacrylamide gels in order to compare the relative mobilities of the genes in the two gel systems (Figure 8). The order of genes on polyacrylamide and agarose was identical. Although segments 1, 2, 4, 5, 7, 8, and 9 exhibited a slight degree of contamination with the next smallest segments (Figure 8), additional gel purification cycles were unnecessary because the concentration of target segment was much greater than the minor contaminant. Segments were hybridized individually to genome profiles of Palyam serogroup viruses which had been transferred from polyacrylamide to Zeta-probe membrane. One of ten blot hybridization is shown (Figures 9a and 9b), where it is evident that segment 10 of Palyam virus hybridized only to segment 10 in each isolate. The remaining nine segments hybridized only to one segment in the other viruses (data not shown). The order of segments 5 and 6 of Bunyip Creek, D'Aguilar, Kasba, and Petevo in polyacrylamide was reversed relative to Palyam (data not shown). Palyam gene 6 exhibited a low level of cross-hybridization with its cognates, with the exception of CSIRO Village where the level of cross-hybridization was much stronger (data not shown).

Segment 2 of Palyam virus hybridized with segment 2 of CSIRO Village, but it did not hybridize with segment 2 of any other isolates (Figures 10a, 10b, 11a, and 11b). Since CSIRO Village was the only isolate which cross-reacted with Palyam in neutralization tests (YARU Annual Report 1983), segment 2 may encode the neutralization antigen of the Palyam serogroup. Since Vellore genes 2 and 3 were comigrant in polyacrylamide and the Palyam gene 2 probe was contaminated with gene 3 (Figures 8; 10a, lane B; and 10b, lane B), it was difficult to determine whether gene 2 of Palyam cross-hybridized to gene 2 of Vellore (Figure 10a, lane J). However, hybridization was not detectable for Vellore gene 2 (data not shown) when Palyam dsRNA was hybridized to Vellore genes which had been separated on agarose (Figure 12).

Sequence Homology of the Ten Palyam Genes to their Cognates. Hybridization of Palyam genomic RNA to the polyacrylamide gel profiles of the other isolates is represented in figures 11a and 11b. Since pCp labeling of genomic dsRNA yields uniformly labeled segments, dsRNA genes whose sequences are closely related to their counterparts in the probe exhibit stronger hybridization signals than those which share less sequence homology with their pCp labeled cognates. For example, a comparison of the levels of hybridization between the segments which correspond to gene 6 of Palyam (genes 6 of Marrakai, Abadina, and CSIRO Village; and genes 5 of D'Aguilar, Bunyip Creek, Petevo, and Kasba) confirmed that Palyam gene 6 was more closely related to its cognate in CSIRO Village than to its cognates in the other viruses (Figure 11a, lane D).

Table 18 summarizes the relative levels of relatedness of Palyam virus genes to their cognates in the other members of the serogroup. The scoring of genes 4 and 10 of Vellore was confirmed by comparing figure a to a lighter exposure of the same blot (not shown). Genes 5, 7, and 9 consistently exhibited the strongest hybridization signals, and therefore, they appeared to be the most conserved genes of the serogroup. In general, genes 1, 3, 6, and 8 exhibited less relatedness to their cognates in Palyam than did genes 4 and 10. Vellore was the only strain in which segments 1, 3, and 8 exhibited strong signals, whereas CSIRO Village was the only strain in which gene 6 exhibited a strong signal.

Genome Profiles of Palyam Serogroup Isolates in Agarose. Although the cognate genes of Palyam serogroup viruses exhibit variability in their apparent molecular weights in polyacrylamide (YARU Annual Report 1983), their molecular weights in agarose were very similar (Figure 12). Denaturation of dsRNA segments with glyoxal prior to electrophoresis in agarose did not change their relative molecular weights (data not shown). Since comigration of genome segments on agarose gels was not observed, hybridization of genome segments transferred in 10X SSC from agarose may be used to confirm positive or negative hybridization of segments that comigrate on polyacrylamide, such as, genes 2 and 3 of Vellore.

These hybridization data and the observed differences in polyacrylamide gel mobilities (YARU Annual Report 1983) suggest that the sequences of cognate genes of members of the Palyam serogroup have

diverged. Yet, their molecular weights have been maintained. A comparison of the two gel systems emphasizes the influence of secondary structure in the migrational rates of dsRNA segments in polyacrylamide gels, and it demonstrates the usefulness of polyacrylamide profiles as a diagnostic tool for dsRNA viruses.

The gene encoding the reovirus type-specific antigen was identified under conditions identical to those used in this study (see above). Thus, gene 2 may encode the neutralization antigen(s) of Palyam serotypes with a possible minor contribution of gene 6 to the serological reactivity. The sequence variability of gene 2 may be due to reassortment of genome segments with viruses outside the Palyam serogroup. Alternatively, gene 2 may have evolved more rapidly than the nine other genes without the contribution of new genes from outside the serogroup. In the latter case, gene 2 might be expected to exhibit a decreased, but significant level of homology. Nevertheless, any detectable homology may also be a direct result of the necessity for functional similarity, regardless of the origin of the gene.

The genetic relatedness between Palyam and the other viruses is striking in view of the fact that they have been isolated in Africa, Asia, and Australia over the past twenty to twenty-five years (YARU Annual Report 1983). Yet, the number and identity of highly related genes between viruses varies. Palyam and CSIRO Village were initially grouped into a single antigenic complex (YARU Annual Report 1983) on the basis of neutralization tests. Yet, Palyam is also closely related to Vellore when all ten genes are compared. Further studies using reciprocal RNA-RNA blot hybridization will indicate which strains are most closely related within this serogroup, and may provide insight as to their phylogeny.

These data are currently in press in Virology.

Table 18
Relatedness of Palyam Genes[a]

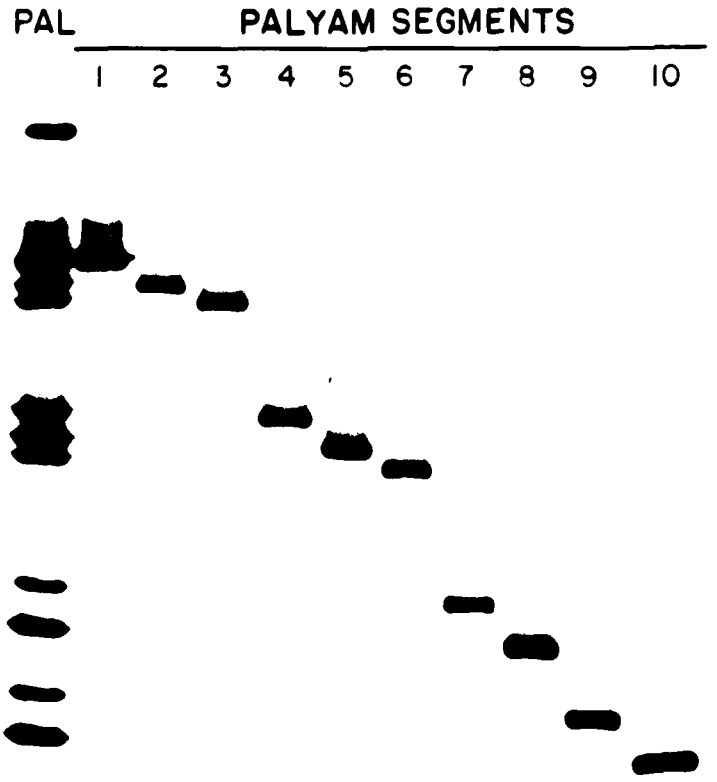
Segment	Kasba (KAS)	Petevo (PET)	Marrakai (MAR)	Abadina (ABA)	CSIRO Village (CV)	Bunyip Creek (BC)	D'Aguilar (DAG)	Vellore (VEL)	Palyam (PAL)
1	+[b]	+	+	+	+	+	+	PAL	PAL
2	-	-	-	-	+	-	-	-	PAL
3	+	+	+	+	+	+	+	PAL	PAL
4	PAL	PAL	PAL	+	PAL	PAL	PAL	+[c]	PAL
5	PAL	PAL	PAL	PAL	PAL	PAL	PAL	PAL	PAL
6	+	+	+	+	PAL	+	+	+	PAL
7	PAL	PAL	PAL	PAL	PAL	PAL	PAL	PAL	PAL
8	+	+	+	+	+	+	+	PAL	PAL
9	PAL	PAL	PAL	PAL	PAL	PAL	PAL	PAL	PAL
10	PAL	+	PAL	+	PAL	PAL	PAL	+[c]	PAL

[a]Membranes containing the genome profiles of each of the isolates were hybridized to 1 - 2 ug (approximately 10^6 CPM) of [5'-^{32}P]-pCp labeled genomic RNA from Palyam as described in the text. Hybridization conditions were 52 C, 50% formamide, 5X SSC.

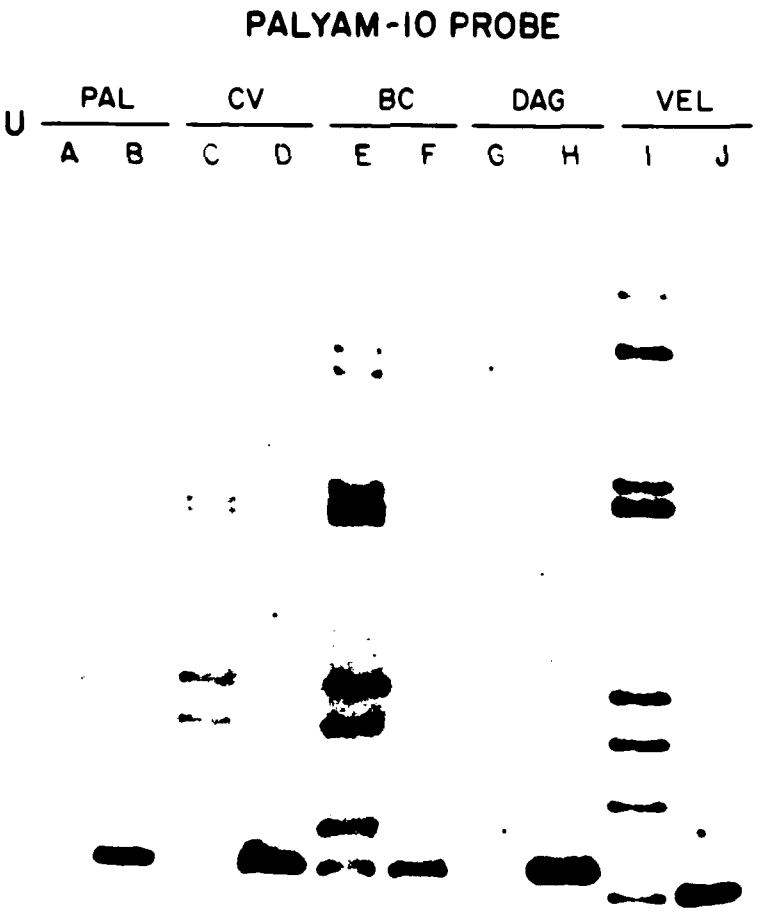
[b]The genes which exhibited strong hybridization signals are designated PAL, those exhibiting faint signals are indicated by a +, those exhibiting no signal are denoted by a -. When two genes were comigrant in polyacrylamide gels, scoring of hybridization signals was confirmed by comparison of the blot shown in Figure to hybridizations using individual labeled segments as probes.

[c]The scoring of genes 4 and 10 of Vellore was confirmed by comparing the blot shown in Figure 11 to a lighter exposure of the same blot as described in the text.

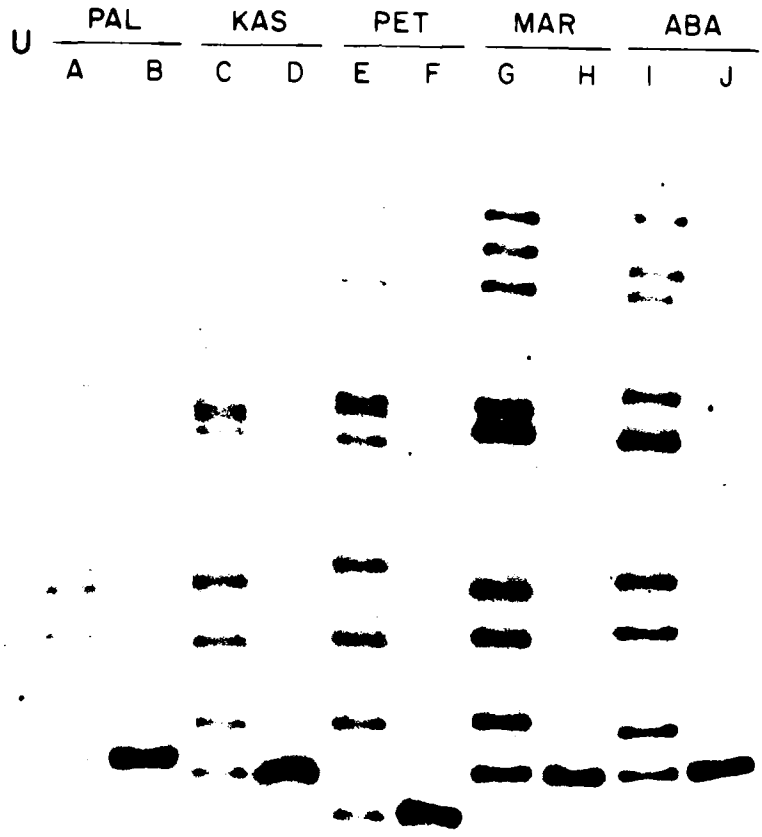
Figure 8. Autoradiogram of end-labeled, purified Palyam virus segments. Genomic dsRNA from Palyam virus was electrophoresed in 0.83% (w/v) agarose and the genome segments were isolated. The individual segments were [5′-^{32}P]-pCp labeled and electrophoresed through 10% polyacrylamide gels to compare the relative mobilities of dsRNA segments between agarose and polyacrylamide gels. The lanes are Palyam virus dsRNA profile (PAL) and Palyam segments 1-10 isolated from agarose, respectively.



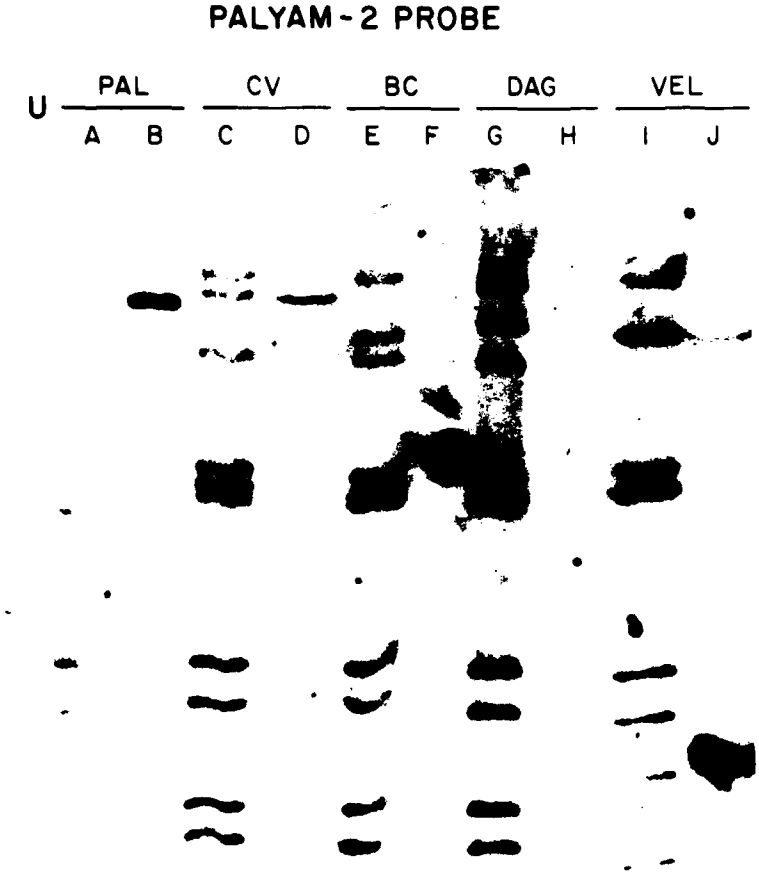
Figures 9 a and b. Autoradiogram depicting identification of genes corresponding to segment 10 of Palyam virus. Segment 10 was isolated from a low melting point agarose gel, [5′-^{32}P]-pCp labeled and hybridized to the genome profiles of nine Palyam serogroup viruses. [5′-^{32}P]-pCp labeled dsRNA from each virus was electrophoresed next to the respective unlabeled dsRNA. Comparison of labeled and unlabeled lanes allows identification of genes in the unlabeled lanes which hybridized with the probe. In Figure 9a, lanes are uninfected cellular control (U), pCp labeled (A) and unlabeled (B) Palyam (PAL), pCp labeled (C) and unlabeled (D) CSIRO Village (CV), pCp labeled (E) and unlabeled (F) Bunyip Creek (BC), pCp labeled (G) and unlabeled (H) D'Aguilar (DAG), and pCp labeled (I) and unlabeled (J) Vellore (VEL). Although the D'Aguilar marker segments are not visible in lane G, the D'Aguilar segment exhibiting a positive signal in lane H had a mobility identical to segment 10 of Bunyip Creek and CSIRO Village. Segment 10 has been shown previously to exhibit identical mobilities for these three viruses (YARU Annual Report 1983). In Figure 9b, lanes are uninfected cellular control (U), pCp labeled (A) and unlabeled (B) Palyam (PAL), pCp labeled (C) and unlabeled (D) Kasba (KAS), pCp labeled (E) and unlabeled (F) Petevo (PET), pCp labeled (G) and unlabeled (H) Marrakai (MAR), and pCp labeled (I) and unlabeled (J) Abadina (ABA).



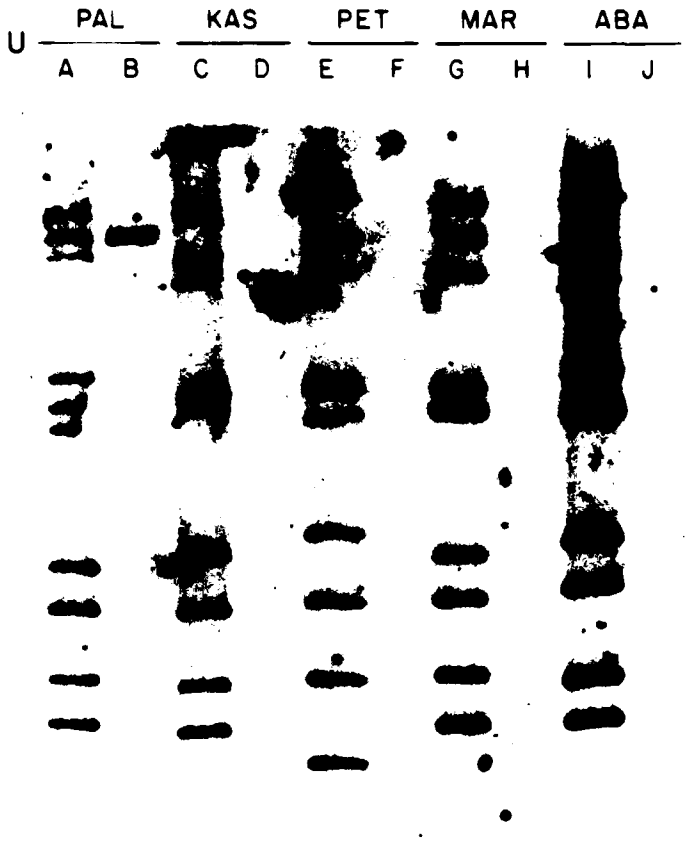
PALYAM - 10 PROBE



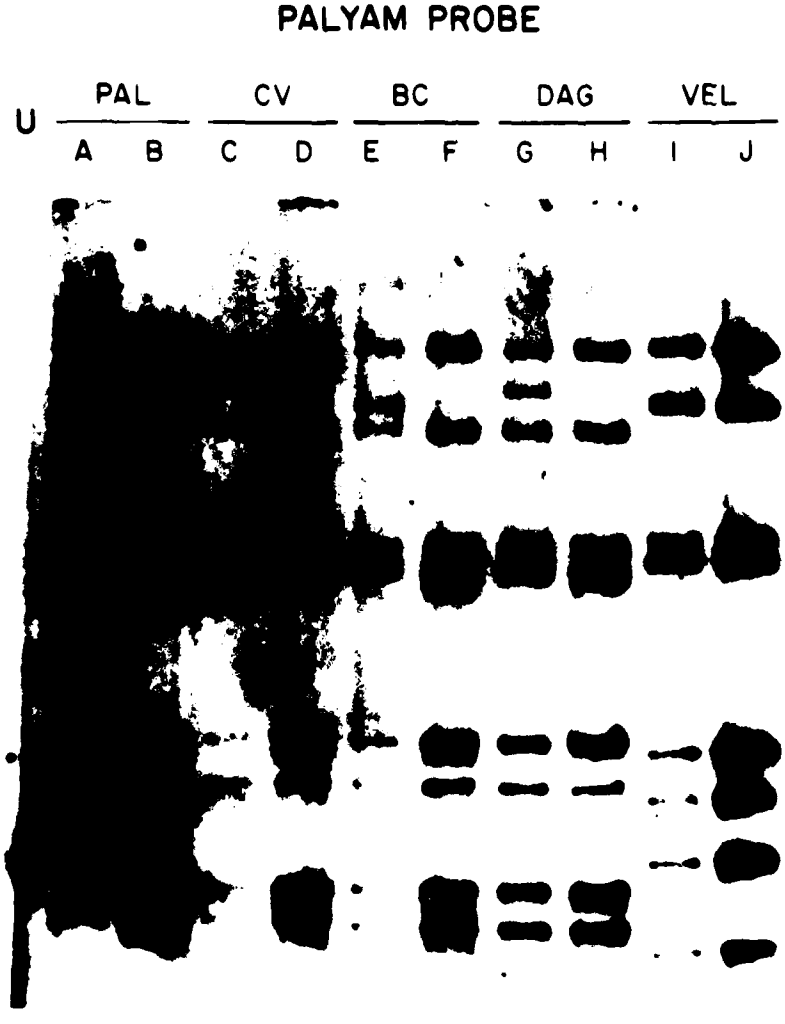
Figures 10a and b. Autoradiogram depicting hybridization of Palyam segment 2 to the profiles of Palyam serogroup isolates. Segment 2 was isolated from a low melting point agarose gel, [5′-^{32}P]-pCp labeled and hybridized to the genome profiles of nine Palyam serogroup viruses as described in the legend of Figure 9.



PALYAM - 2 PROBE



Figures 11a and b. Autoradiogram depicting hybridization of all ten Palyam virus genes to their counterparts in the Palyam serogroup viruses. Total genomic dsRNA from Palyam virus was end-labeled with [5′-^{32}P]-pCp and hybridized to the genome profiles of nine members of the serogroup as described in the legends of Figures 9 and 10.



PALYAM PROBE

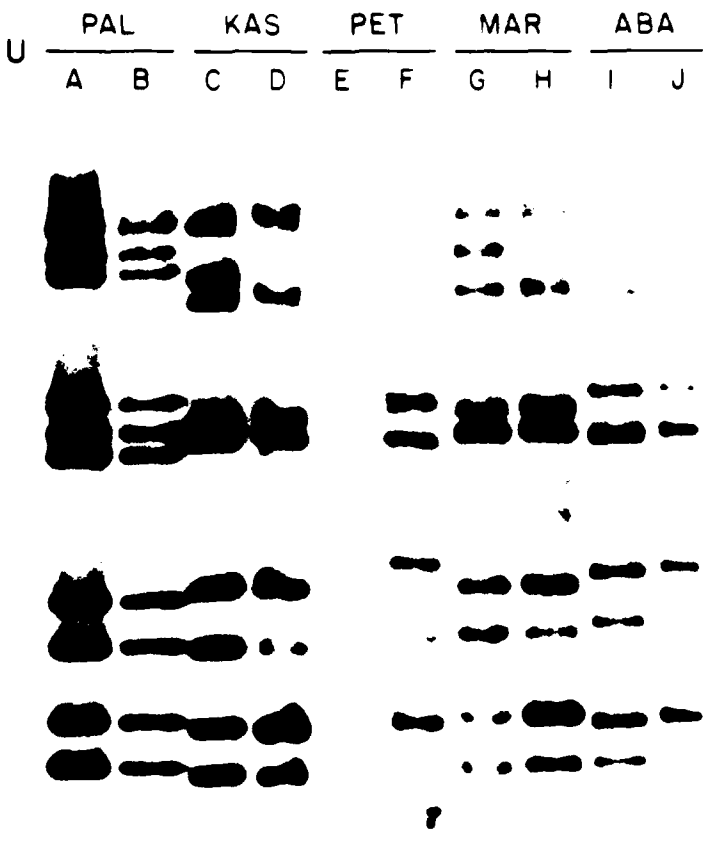
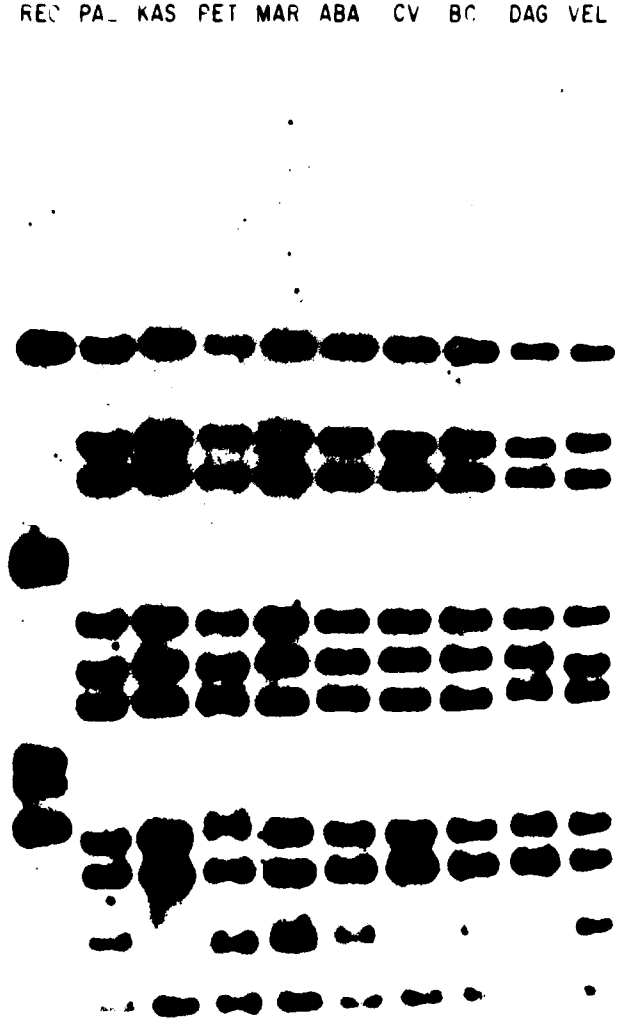


Figure 12. Autoradiogram depicting resolution of the dsRNA genomes of Palyam serogroup isolates in a 1% agarose gel. Lanes are Reovirus 3 (REO), Palyam (PAL), Kasba (KAS), Petevo (PET), Marrakai (MAR), Abadina (ABA), CSIRO Village (CV), Bunyip Creek (BC), D'Aguilar (DAG), Vellore (VEL).



Stability and use of 4G2-4-15 monoclonal as a universal capture antibody in ELISA for rapid diagnosis of flavivirus infections (J.G. Olson, L. Lee and R.E. Shope). The reference center at Yale maintains mouse brain antigens of over 60 flaviviruses. In an attempt to develop an ELISA to use these existing stocks without further purification of the antigens, a universal flavivirus coating antibody was sought. The group-reactive flavivirus monoclonal antibody, 4G2-4-15 seemed to meet the needs of the test. This monoclonal antibody was developed by Gentry et al. (Am. J. Trop. Med. & Hyg. 31:548-555, 1982), who showed it to be of the IgG2a class which fixed complement, and reacted with the hemagglutinin fracton of virus preparations. This fraction contains the envelope glycoprotein.

The test for antibody detection was designed with the 4G2 monoclonal antibody attached to the solid phase to capture the mouse brain antigen. The wells were washed, the test human serum was added, then anti-human peroxidase conjugate, followed by ABTS substrate. The coat antibody was diluted in PBS pH 7.4 and the wells were coated at 4°C for at least 16 h. All other dilutions ere made in PBS with 0.05% Tween-20, 5% normal horse serum, and dextran sulfate 50 micrograms/L. Incubations were 1 h at 37°C. Volumes were constant at 100 microliters. The O.D. was determined at 414 lambda in a Titertech Multiscan ELISA reader.

The stability of the 4G2 antibody on the solid phase was tested. Plates were coated at 1:400 dilution of 4G2 antibody, held at 4°C for 16 h, then stored in different ways (Table 19). Dengue-2 mouse brain antigen was used 1:50 with acute and convalescent sera from patients who had dengue-2 infections. The coating antibody was stable for at least 52 days at 4°C, stored wet in a humidified chamber. Companion plates were air-dried, then either stored at -70°C or dried further under vacuum for 5 h and sealed in plastic in dry nitrogen gas atmosphere. The vacuum-dried plates were held at 4°C (Table 20). There was no difference in the function of these plates under each of these storage conditions.

An additional experiment tested the stability of the coating antibody stored in plastic bags at 25°C and 37°C for one month (Table 21). Although there may have been some loss of reactivity, plates stored under these adverse conditions could still be used.

In a final stability experiment, plates were coated for 16 h, then dengue-2 mouse brain antigen was added for 1 h at 37°C. The wells were then dried under vacuum and kept in plastic bags under dry nitrogen gas atmosphere (Table 22). After storage at 4°C for 1 month, the test functioned well. It also functioned after plates were kept at 25°C, although there may have been some loss in sensitivity. Storage of antigen at 37°C on the plates for one month, however, was not satisfactory.

These results indicated that the 4G2 monoclonal antibody was stable on polystyrene plates for weeks (and probably much longer) in several forms, wet at 4°C, air-dried at -70°C and vacuum-dried under dry nitrogen gas at various temperatures including 37°C. It should be feasible, therefore, to send coated plates by mail anywhere in the world. In addition, coated plates with antigen attached can be vacuum dried and are relatively stable at 25°C, but not 37°C.

Table 19

Stability of monoclonal antibody 4G2-4-15 as ELISA capture antibody coated on 96-well plates and stored at 4C in liquid phase

	Reciprocal of antibody titer			
	Patient A		Patient B	
Day	Acute	Convalescent	Acute	Convalescent
0	Negative	≥6400	Negative	≥6400
7	Negative	≥6400	Negative	≥6400
14	Negative	≥6400	Negative	≥6400
21	Negative	3200	Negative	1600
30	Negative	3200	Negative	3200
52	Negative	≥6400	Negative	≥6400

Table 20

Comparison of stability of capture antibody stored in different forms

		Liquid phase 4C	-70C dry	Lyophilized 4C
one month	A acute:	Negative	Negative	Negative
	conval:	3200	≥6400	≥6400
	B acute:	Negative	Negative	Negative
	conval:	3200	≥6400	≥6400
two months	A acute:	Negative	Negative	Negative
	conval:	≥6400	≥6400	≥6400
	B acute:	Negative	Negative	Negative
	conval:	≥6400	≥6400	≥6400

Table 21

Stability of capture antibody 4G2-4-15 dried in vacuum and stored at 25C and 37C

	Patient A		Patient B	
	Acute	Convalescent	Acute	Convalescent
4C	<200	≥6400	<200	>6400
25C	<200	800	<200	1600
37C	<200	1600	<200	1600

Table 22

Stability of capture antibody 4G2-4-15 and dengue-2 antigen dried in vacuum and stored at 25C and 37C

	Patient A		Patient B	
	Acute	Convalescent	Acute	Convalescent
4C	<200	≥6400	<200	≥6400
25C	<200	≥6400	<200	1600
37C	<200	<200	<200	<200

Attempts to demonstrate that 4G2 can serve as a universal capture antibody for flavivirus antigens were limited because we had human sera with neutralizing antibody to only a few flaviviruses. Six antigens were tested: dengue-2, Japanese encephalitis, St. Louis encephalitis, West Nile, Zika, and yellow fever. All antigens functioned well with the 4G2 antibody. The human sera were from Cuba (dengue-2), United States (yellow fever and Japanese encephalitis vaccinee), Panama (St. Louis encephalitis), Israel (West Nile), and Indonesia (Zika). Table 23 shows the results of ELISA using five of these antigens. The 4G2 antibody has a wide spectrum of reactivity as a flavivirus capture antibody. It was not the purpose of these experiments to determine the specificity of the ELISA among flaviviruses; however it is apparent that some sera are broadly cross-reacting, especially sera from persons who have secondary type antibody response to flaviviruses. In these cases ELISA cross-reacts in a manner analagous to the HI test.

Monoclonal antibodies as polyvalent alphavirus grouping reagents (J.M. Meegan, J.G. Olson, D. Winograd and R.E. Shope). As part of a collaborative project of a working group on monoclonal antibodies of the Subcommittee on Interrelationships of Catalogued Arboviruses (SIRACA), candidate grouping alphavirus monoclonal antibodies were supplied for testing by Dr. Alan Schmaljohn of the University of Maryland and by Dr. John Roehrig of CDC, Fort Collins. The reagents were tested by IFA and HI with a battery of alphaviruses representing nearly all known types and subtypes. The results are shown in Tables 24 through 28.

Six of the monoclonal antibodies prepared from mice immunized with both Sindbis and Semliki Forest viruses were reactive with nearly all alphaviruses by IFA and HI. Testing was not completed with the 3 candidates from mice immunized with EEE or WEE viruses, but they reacted by HI with 7 antigens tested. These antibodies, either alone or in combination should be very useful for initial identification of alphaviruses and perhaps as coating antibody for ELISA or as conjugates in the ELISA and FA test.

ELISA to detect antibody in seabirds (John J. Oprandy). An ELISA test was developed to assay gull sera for the presence of antobodies to Great Island complex viruses. The indirect sandwich technique (viral antigen + unknown serum + mouse antigull ascitic fluid + enzyme conjugated goat anti-mouse) was found to be complex specific. Sucrose-acetone extracted Great Island infected mouse brain was used as plate substrate. Antisera to GI complex viruses broadly cross reacted while antisera to other Kemerovo group complexes did not (data not shown). Gull sera were extracted with kaolin to reduce non-specific binding. A gull serum-specific mouse immune ascitic fluid was generated by intra-peritoneal inoculation of mice with ammonium sulfate precipitated gull sera and Freunds complete adjuvant.

A total of 94 gull sera was tested by ELISA (Table 29). All 64 samples from gull chicks and immatures were negative. Five of 22 (22.7%) adults, all of which were Herring Gulls, were positive. The five positive sera will be assayed by PRNT for virus specific antibody.

A total of 261 ticks was collected and divided into 38 pools. Twenty-nine pools were Dermacentor variabilis from Martha's Vineyard and Natucket. The remaining pools were as follows: 1-I. dammnini (Martha's Vineyard), 3- I.

Table 23

Use of 4G2-4-15 monoclonal antibody in ELISA to capture antigens of mosquito-borne flaviviruses

	Antigen				
Human sera immune to:	JE	SLE	WN	Zika	YF
Dengue-2 (Cuba)	200	400	400	0	1600
JE/YF (USA)	3200	0	6400	3200	$\geq$12,800
SLE (Panama)	200	3200	800	800	1600
WN (Israel)	0	0	800	0	200
Zika (Indonesia)	3200	$\geq$12,800	6400	6400	$\geq$12,800
YF (USA)	800	1600	800	800	3200

Table 24

Immunofluorescence reactions with alphaviruses using monoclonal antibodies prepared against Sindbis/Semliki Forest viruses by Dr. Alan Schmaljohn, University of Maryland

	Antigens							
Antibody	BEB	CHIK	EEE	GET	MAY	ONN	RR	SAG
K07	16000	16000	32000	16000	32000	32000	32000	500
K19	16000	8000	8000	8000	8000	16000	16000	1000
K37	12800	12800	25600	12800	12800	12800	25600	400
K42	12800	12800	25600	12800	51200	25600	25600	1600
K43	32000	8000	32000	>1000	32000	32000	32000	1000*
S16	>1000	>1000	>1000	>1000	>1000	>1000	>1000	0*
K31	0	>100	>1000	>1000	>1000	0	>1000	0
K54	0	>100*	0	0	0	0	0	0
K18	0	0	0	0	0	0	0	0
K22	0	0	0	0	0	0	0	0
K04	0	0	0	0	0	0	>1000	0
K34	0	0	0	0	0	0	0	0
K45	0	0	0	0	0	0	0	0
S49	0	0	0	0	0	0	0	0
S50	0	0	0	0	0	0	0	0
S33	0	0	>1000	0	0	0	0	0
S38	0	0	0	0	0	0	0	0

* weak reaction; 0 = <100.

Table 25

Immunofluorescence reactions with alphaviruses using monoclonal antibodies prepared against Sindbis/Semliki Forest viruses by Dr. Alan Schmaljohn, University of Maryland

	Antigens						
Antibody	SF	SIN	VEE	WEE	WHA	NDU	MID
K07	16000	32000	32000	32000	16000	>1000	0
K19	8000	16000	8000	16000	8000	>1000	0
K37	12800	12800	12800	25600	12800	>1000	0
K42	12800	>102400	25600	51200	25600	>1000	0
K43	16000	64000	8000	16000	8000	>1000	0
S16	>100	>1000	>1000	>1000	>1000	0	>1000
K31	>100*	>1000	>1000	>1000	>1000	0	0
K54	0	>100	0	>1000	0	0	0
K18	>100*	0	0	0	0	0	0
K22	0	>1000*	0	0	0	0	0
K04	>1000	0	0	0	0	0	>1000
K34	>100	0	0	0	0	0	0
K45	0	0	0	0	0	>1000	0
S49	0	>1000	0	0	0	0	0
S50	0	>1000	0	0	0	0	0
S33	0	>1000	0	>1000	0	0	0
S38	0	>1000*	0	>100	>1000	0	0

* weak reaction; 0 = <100.

Table 26

Hemagglutination-inhibition reactions with alphaviruses using monoclonal antibodies prepared against Sindbis/Semliki Forest viruses by Dr. Alan Schmaljohn, University of Maryland

	Antigens							
	BEB	CHIK	EEE	GET	MAY	ONN	RR	SAG
Units	4	4	4	4	4	4	4	8
Antibody								
K07	1600	3200	12800	>102400	16000	51200	25600	1600
K19	800	3200	1600	51200	400	25600	6400	400
K37	1600	3200	3200	51200	1600	51200	25600	800
K42	12800	1600	12800	51200	1600	51200	51200	6400
K43	1600	3200	6400	51200	1600	51200	25600	3200
S16	3200	800	1600	12800	1600	12800	12800	400
K31	400	3200	800	>102400	200	12800	12800	400
K54	100	0*	0	0	0	0	0	0
K18	0	0	0	0	0	0	0	0
K22	0	0	0	0	0	0	0	0
K04	0	0	0	0	0	0	0	0
K34	0	0	0	0	0	0	0	0
K45	0	0	0	0	0	0	0	0
S49	0	0	0	0	0	0	0	0
S50	0	0	0	0	0	0	0	0
S33	0	0	0	0	0	0	0	0
S38	0	0	0	0	0	0	0	0

*0 = <50.

Table 27

Hemagglutination-inhibition reactions with alphaviruses using monoclonal antibodies prepared against Sindbis/Semliki Forest viruses by Dr. Alan Schmaljohn, University of Maryland

	Antigens							
	SF	SIN	VEE	WEE	WHA	NDU	MID	AURA
Units	4	4	1	4	2	2	8	2
Antibody								
K07	6400	12800	6400	256000	512000	>102400	<100	12800
K19	1600	3200	6400	512000	512000	>102400	<100	6400
K37	3200	12800	6400	12800	512000	>102400	<100	12800
K42	12800	25600	6400	12800	51200	>102400	<100	25600
K43	6400	51200	25600	51200	>102400	>102400	800	12800
S16	3200	25600	12800	64000	128000	12800	1600	3200
K31	400	3200	3200	64000	128000	>102400	800	3200
K54	0*	25600	0	256000	<500	0	800	0
K18	0	0	0	0	0	0	<100	0
K22	0	0	0	0	0	0	<100	0
K04	12800	0	0	0	0	0	400	0
K34	6400	0	0	0	0	0	0	0
K45	6400	0	0	0	0	0	0	0
S49	0	1600	0	0	2000	0	0	0
S50	0	800	0	0	500	0	0	0
S33	0	6400	0	2000	500	0	0	0
S38	0	50	0	<500	1000	0	0	400

*0 = <50.

Table 28

Hemagglutination-inhibition reactions with alphaviruses using monoclonal antibodies prepared against EEE and WEE viruses by Dr. John Roehrig, CDC, Fort Collins, Colorado

	Antigens						
	GET	WHA	ONN	VEE	WEE	MID	RR
Units	4	2	4	1	4	8	4
Antibody							
EEE 1A4B-6	12800	>1000000	51200	25600	>1000000	12800	51200
WEE 2A2C-3	3200	>1000000	6400	3200	512000	800	6400
WEE 2B6B-2	12800	>1000000	25600	25600	>1000000	<100	25600

dentatus (Penekese), 3- H. leporispalustris (Penekese) and 2- I uriae (Quebec). Twelve of the D. variabilis and all of the other species pools were tested. No viruses were isolated. An exhaustive search of gulls, their nests and the grounds of their colonies did not reveal the presence of any I. uriae ticks.

These observations are interpreted to indicate that previously infected seabirds have migrated into the Northeast but as of yet there may be no local infections in gull colonies of southern New England. The latter point may be a result of the vector, I. uriae, not being present in this area.

Table 29

Prevalence of Great Island virus complex antibody in Gull populations

	Island			Total
	M. Vineyard	Nantucket	Penekese	
Chicks	0/25	0/36	0/3	0/64
Immature		0/8		0/8
Adults		4/21 (19.0%)*	1/1	5/22 (22.7)
TOTAL	25	65	4	94**

*Positives were judged as having a difference between positive and negative antigen greater than 3 standard deviations from the mean of negative control sera.
** 39 Black-backed Gulls (all chicks), 55 Herring Gulls.

ELISA using PAGE purified St. Louis Encephalitis virus proteins (E. McClure, J. Oprandy, A.J. Main and R.E. Shope). Enzyme-linked immunosorbent assay has been used for the detection of antibodies to a number of viruses. Flaviviruses have been shown to be cross reactive by ELISA making it difficult to determine the infecting virus (Meegan, J., YARU Annual Report, 1980, p. 71). Antisera to Yellow Fever, St. Louis Encephalitis (SLE) and Ilheus viruses are broadly cross-reactive in the ELISA test when total viral antigen is used as plate substrate in an indirect test (viral antigen + human sera + enzyme conjugated anti-human antibody).

A project was initiated to develop a SLE specific ELISA using SDS-PAGE purified viral proteins as plate substrate. SLE virus, grown in Vero cells, was purified by ultrafiltration in Centricon tubes and later eluted. Virus particles were disrupted in SDS/2-mercaptoethanol and viral proteins electrophoresed in 10% Laemmli gels. Proteins were then transferred to a nitrocellulose membrane by capillary diffusion in replicates. An ELISA was then performed on these replicates using different flavivirus antisera.

Preliminary data indicate that there are viral protein bands which do not cross react with antisera to other flaviviruses. Proteins will next be eluted from the gel matrix and used as plate substrate in an attempt to develop a SLE virus specific ELISA.

V. COLLECTION OF LOW PASSAGE ARBOVIRUS REFERENCE STRAINS (R.B. Tesh)

In collaboration with the Subcommittee for the Collection of Low Passage Arbovirus Strains (SCLAS) of the American Committee on Arthropod-Borne Viruses (ACAV), an attempt has been made to establish a collection of low passage strains of selected arboviruses of public health importance. Previous reports about the collection were given in the 1981, 1982 and 1983 Annual Reports. During 1984, stocks of 93 more virus strains were prepared and lyophilized. These agents as well as their origin and passage history are listed in Table 30.

During 1984, YARU also received a large number of viruses from the collection of the late Dr. William F. Scherer. Most of these samples are strains of Venezuelan equine and Japanese encephalitis viruses collected over a 30-year period during Dr. Scherer's extensive field work. Many are prototype or original isolates and represent valuable reference material. These viruses were obtained through the kindness of Dr. Robert W. Dickerman, Cornell University School of Medicine.

It is hoped that interested persons working in arbovirology will continue to submit samples of low passage virus strains from different geographical locations and time periods. We intend to create a data file with pertinent information on each virus in the collection. This information as well as the lyophilized virus stocks will be available to interested investigators at no cost. It is anticipated that this collection will prove to be an invaluable reference resource for future comparative studies on viral genetics, biochemistry, pathogenicity and antigenic relationships.

VI. DISTRIBUTION OF REAGENTS, WHO COLLABORATING CENTRE FOR REFERENCE AND RESEARCH (R.E. Shope, A. Main, R.B. Tesh, S. Buckley, J. Olson, G.H. Tignor)

The equivalent of 970 ampoules of arbovirus reagents were distributed from the WHO Centre to laboratories in 22 countries during 1984. This total consisted of 206 ampoules of virus stock, 499 ampoules of virus antigen, and 265 ampoules of mouse immune ascitic fluid or sera. Of the viruses and antibody distributed, there were represented 185 different arboviruses.

During 1984, the equivalent of 1,446 ampoules of arbovirus reagents was referred to this Centre from laboratories in 31 different countries. The referrals consisted of 192 ampoules of viruses (Table 31) and 1,254 sera received for diagnosis and arbovirus antibody survey testing.

Eight different cell lines and colonized insects were distributed in 1984 to 11 laboratories.

Table 30

Low Passage Virus Strains - 1984

Virus	Strain	Passage	Source	Locality	Date
La Crosse	78V 13193	SM #1, Vero #2	Aedes triseriatus(larvae)	North Carolina	1978
VEE (1-D)	71D 1394	SM #1, C6/36 #1	Mixed mosquitoes	Peru	1971
VEE (1-D)	310979	C6/36 #1	Sentinel hamster	Puerto Boyaca,Colombia	June 1974
VEE (1-E)	68U 200	C6/36 #1	Sentinel hamster	Avellana,Guatemala	May 1977
VEE (1-E)	64A 87	SM #1, C6/36 #1	Culex opisthopus	Sontecomapan,Mexico	1964
Dengue-1	1186	Mosq.#2,C6/36 #1	human serum	Jakarta,Indonesia	1977
Dengue-1	1236	"	"	Jakarta,Indonesia	1978
Dengue-1	1298	"	"	Mexico	1980
Dengue-1	1318	"	"	Puerto Rico	1981
Dengue-1	1335	"	"	Colombo,Sri Lanka	1981
Dengue-1	1344	"	"	Mexico	1982
Dengue-1	1351	"	"	Colombia	1982
Dengue-1	1378	"	"	Mexico	1983
Dengue-1	1412	"	"	Mexico	1983
Dengue-1	1413	"	"	Haiti	1983
Dengue-2	1232	Mosq.#2,C6/36 #2	human serum	Jakarta,Indonesia	1978
Dengue-2	1251	"	"	Tonga	1974
Dengue-2	1268	"	"	Jogyakarta,Indonesia	1978
Dengue-2	1328	"	"	Puerto Rico	1977
Dengue-2	1329	"	"	Jamaica	1982
Dengue-2	1334	"	"	Colombo,Sri Lanka	1981
Dengue-2	1349	"	"	Upper Volta	1982
Dengue-2	1353	"	"	Colombo,Sri Lanka	1982
Dengue-2	1408	"	"	Jamaica	1983
Dengue-2	1421	"	"	Mexico	1983

Table 30 (continued)

Low Passage Virus Strains - 1984

Virus	Strain	Passage	Source	Locality	Date
Dengue-3	1245	Mosq.#2,C6/36 #2	human serum	Sleman,Indonesia	1978
Dengue-3	1259	"	"	Jakarta,Indonesia	1978
Dengue-3	1301	"	"	Malaysia	1975
Dengue-3	1309	LLC/MK$_2$#1,Mosq.#2,C6/36 #1	human serum	Bangkok,Thailand	1978
Dengue-3	1325	Mosq. #2, C6/36 #1	"	Colombo,Sri Lanka	1981
Dengue-3	1339	"	"	Puerto Rico	1977
Dengue-3	1359	"	"	Colombo,Sri Lanka	1982
Dengue-3	1363	"	"	Colombo,Sri Lanka	1982
Dengue-4	1228	Mosq. #2, C6/36 #1	human serum	Jogyakarta,Indonesia	1978
Dengue-4	1229	"	"	Jakarta,Indonesia	1976
Dengue-4	1315	"	"	Puerto Rico	1981
Dengue-4	1331	"	"	Puerto Rico	1982
Dengue-4	1332	"	"	Puerto Rico	1982
Dengue-4	1385	Vero #1,Mosq.#2,C6/36 #1	"	Boa Vista,Brazil	1982
Dengue-4	1411	Mosq. #2, C6/36 #1	"	San Salvador,El Salvador	1983
Dengue-4	1414	Mosq. #2, C6/36 #1	"	Mexico	1983
Dengue-4	1415	"	"	Mexico	1983

Table 30 (continued)

Low Passage Virus Strains - 1984

Virus	Strain	Passage	Source	Locality	Date
EEE	R-35108	C6/36 #1	horse brain	Three Rivers,Michigan	1980
SLE	FL 79-411	C6/36 #1	Culex nigripalpus	Lee County,Florida	1979
Yellow fever	1362/77	C6/36 #1	human serum	Mutucana,Peru	1977
WEE	NM5-7ET	C6/36 #1	Aedes vexans	Rancho de Albiquin,New Mexico	1965
Highlands J	NJO-111D	C6/36 #1	Culeseta melanura	S.R. Game Farm,New Jersey	1960
LaCrosse	prototype	C6/36 #1	human brain	LaCrosse, Wisconsin	1960
LaCrosse	78134	C6/36 #1	human brain	Wisconsin	1978
JE	KE-105/83	C6/36 #1	human brain	Kampangphet, Thailand	1983
JE	KE-094/83	C6/36 #1	human brain	Kampangphet, Thailand	1983
JE	B-1080/83	C6/36 #1	pig serum	Choomporn, Tahiland	1983
Dengue-1	PUO-359	C6/36 #1	human serum	Bangkok, Thailand	1980
Dengue-3	D83-144	C6/36 #1	human serum	Bangkok, Thailand	1983
JE	KP0035-114CT	C6/36 #2	Culex tritaeniorhynchus	Kampangphet, Thailand	1982
EEE	70U1104	Vero #1	sentinel hamster	Iquitos, Peru	1970
VEE-ID	V-209A	SM #2,Vero #2	sentinel mouse	Santander, Colombia	1960
VEE-ID-E	CoAn 59145	? ,Vero #1	sentinel hamster	Santander, Colombia	1970
VEE-IA	CoAn 5384	SM #2,CEC #1, Vero#1	horse	Carmelo, Colombia	1967
VEE-V	CaAn 410d	SM #3,VERO #1	Psaracolus decumanus	Tonate, French Guyana	1973
VEE-IA	71D 1252	SM #1,Vero #1	Mosquito pool	Iquitos, Peru	1971
VEE-ID-E	75D 143	SM #2,Vero #1	Mosquito pool	Iquitos, Peru	1975
VEE-IV	BeAr 40403	Sm #6,Vero #1	Trichoprosopon digitatum	Belem, Brazil	1961
VEE-IB	69U332	Vero #1	sentinel hamster	La Avellana,Guatemala	1969
VEE-IA	E123/69	SM #1,CEC #1, Vero#1	human	Mara, Venezuela	1969

Table 30 (continued)

Low Passage Virus Strains - 1984

Virus	Strain	Passage	Source	Locality	Date
VEE-IC	CBS1-9	Vero #2	Anopheles triannulatus	Sotillo, Venezuela	1963
VEE-IE	68U 201	Vero #1	sentinel hamster	La Avellana,Guatemala	1968
VEE-ID-E	71D 1249	SM #1,Vero #1	Mosquito pool	Iquitos, Peru	1971
VEE-ID-E	70U 1139	Vero #2	sentinel hamster	Iquitos, Peru	1970
VEE-III	TVL 52049	Vero #2	Zygodontomys brevicauda	Bush-Bush, Trinidad	1963
VEE-IB	541/73	SM #1,CEC #2,Vero #1	human	Guajira, Venezuela	1973
VEE-IA	E1/68	SM #1,CEC #1,Vero #1	human	Guajira, Venezuela	1968
VEE-ID	202330	SM #1,Vero #2	Proechimys semispinosus	Gamboa, Panama	1963
VEE	64U 60	SM #1	sentinel hamster	Santecomapan, Mexico	1964
VEE	83U 12	SM #1	sentinel hamster	Rio de Oro, Colombia	1983
VEE-IC	V-198	SM #1,Vero #2	human serum	Guajira, Colombia	1962
VEE-IC	V-178	SM #1,Vero #2	human	Cundinamarca, Colombia	1961
VEE-IE	63A 216	SM #2,Vero #1	Culex spp. (pool)	Sontecomapan, Mexico	1963
VEE-ID	3880	SM #2,Vero #3	human serum	Canito, Panama	1961
VEE-III	Fe 37C	SM #6,Vero #1	Culex spp. (pool)	Florida, USA	1963
VEE-ID-E	Tumaco An9004	SM #3,Vero #1	sentinel hamster	Pacific coast, Colombia	1969
VEE-IE	68U 201	SM #1,Vero #1	sentinel hamster	La Avellana, Guatamala	1968
VEE-IE	71U 338	SM #1,Vero #1	sentinel hamster	Monte Rico, Guatemala	1971
VEE-V	CaAr 4389	SM #4,CEC #1, Vero #1	Culex spp. (pool)	Cabassou, French Guyana	1974
VEE-IE	71U 384	SM #1,Vero #1	sentinel hamster	La Avellana, Guatemala	1971

Table 30 (continued)

Low Passage Virus Strains - 1984

Virus	Strain	Passage	Source	Locality	Date
VEE-IV	BeAr 35645	SM #4,Vero#1	Anopheles nimbus	Belem, Brazil	1961
VEE-IA	52/73	SM #2,VERO #1	burro	La Libertad, Peru	1973
VEE-II	Fe5-47 et	SM #2,Vero #1	Aedes taeniorhynchus	Florida, USA	1965
VEE-IB	6921	SM #2,Vero #1	human serum	Montufar, Guatemala	1969
VSV-New Jersey	Jardin 12-IV	bovine #1,Vero #1		Verocruz, Mexico	1982
EEE	M-649-84	original brain	horse	Waterford, Connecticut	1984
VSV-New Jersey	Ossabaw	Vero #2	feral pig	Ossabaw Island, Georgia	1983

Table 31

Viruses referred to YARU for identification and study, 1984

Country of origin; strain	Source	Information from donor	YARU identification
Argentina			
AG 83-1347	mosquitoes	related to Jurona virus	new vesiculovirus
Australia			
GG668	mosquitoes	Paroo River virus	
Cs 264	Culicoides austropalpalis	unidentified	
Cs 976	Culicoides austropalpalis	unidentified	
Cs 1056	Culicoides austropalpalis	unidentified	
Cs 1196	bovine	D'Aguilar	
Cs 1328	Aedes (Verrallina) spp.	unidentified	
Cs 1342	Culex spp.	? rhabdovirus	
Cs 1358	Culicoides brevitarsis	CSIRO Village	
Cs 1499	Argas robertsi	Unidentified	
Cs 1568	Ixodes eudyptidis	unidentified	
Cs 1625	Culex edwardsi	Sindbis	
DPP 66	bovine	Palyam group	
DPP 158	bovine	Marrakai	
DPP 247	bovine	Bunyip Creek	
RRV	human serum	Ross River	

Table 31 (continued)

Viruses referred to YARU for identification and study, 1983

Country of origin; strain	Source	Information from donor	YARU identification
Central African Republic			
HB83 P30	human serum	West Nile	
HB83 P45	human serum	West Nile	
HB83 P50	human serum	West Nile	
HB83 P53	human serum	West Nile	
HB83 P55	Human serum	West Nile	
Egypt			
Thogoto	ticks	Thogoto, prototype	
France			
Brest An219	shrew	unidentified	
Brest An221	shrew	unidentified	
Brest An227	shrew	unidentified	
Brest ArT598	Ornithodoros maritimus	unidentified	Kemerovo group

Table [illegible]ued)

Viruses referred to YARU [illegible]ification and study, 1983

Country of origin; strain	Source	Information from donor	YARU identification
Korea			
76-118	Apodemus agrarius	Hantaan virus	
80/39	rat	Hantaan-related	
Tchoupitoulas	rat	Hantaan-related	
SR-11	rat	Hantaan-related	
KHF83-61	human blood	Hantaan-related	
Panama			
BT-78	Phlebotomus spp.	VSV-Indiana for early passage collection	
1958 horse	horse	EEE for early passage collection	
900188	horse	EEE for early passage collection	
903866	sentinel chicken	EEE for early passage collection	
903816	human throat swab	VSV-Indiana for early passage collection	
BV-7		SLE for early passage collection	

Table 31 (continued)

Viruses referred to YARU for identification and study, 1983

Country of origin; strain	Source	Information from donor	YARU identification
Philippines			
2123	human serum	Dengue-4 for early passage collection	
2172	human serum	Dengue-2 for early passage collection	
2178	human serum	Dengue-3 for early passage collection	
2304	human serum	Dengue-1 for early passage collection	
not stated	pig blood	Japanese encephalitis for early passage collection	
Senegal			
Unknown virus	Aedes aegypti cell line	virus contaminant	
Sweden			
E16	human specimen	suspect phlebovirus from Swedish tourist after visit to Portugal	
Thailand			
Ke094, 100, 105	human thalamus	Japanese encephalitis strains for early passage collection	
83/1082, 1075, 1080	sentinel pig sera	Japanese encephalitis strains for early passage collection	
CT37-47, 35-114, 39-183	Culex tritaeniorhynchus	Japanese encephalitis strains for early passage collection	
D83-118, PUO-359, PUO-451	human sera	Dengue-1 strains for early passage collection	
D83-126, 191, 265	human sera	Dengue-2 strains for early passage collection	
D83-280, 159, 144	human sera	Dengue-3 strains for early passage collection	
D83-109, 152	human sera	Dengue-4 strains for early passage collection	

Table 31 (continued)

Viruses referred to YARU for identification and study, 1983

Country of origin; strain	Source	Information from donor	YARU identification
USA			
71-1629	ticks	Kenai virus	
Dengue1-4	human blood	dengue strains for use in immunology study	
R-35108		EEE for early passage collection	
FL 79-411		SLE for early passage collection	
1362/77		Yellow fever for early passage collection	
NM5-7ET		WEE for early passage collection	
FE6-17DW		Everglades for early passage collection	
dengue-1	human sera	12 strains for early passage collection	
dengue-2	human sera	10 strains for early passage collection	
dengue-3	human sera	9 strains for early passage collection	
dengue-4	human sera	10 strains for early passage collection	
pool 133	Aedes triseriatus larvae	LaCrosse virus for early passage collection	
pool 81-4	Aedes triseriatus males	LaCrosse virus for early passage collection	
44-71017	human cerebellum	LaCrosse virus for early passage collection	
43-71016	human basal ganglia	LaCrosse virus for early passage collection	

Table 31 (continued)

Viruses referred to YARU for identification and study, 1983

Country of origin; strain	Source	Information from donor	YARU identification
USA continued			
A 78134	human temporal lobe	LaCrosse virus for early passage collection	
Large collection	varied sources	VEE and other strains transferred to YARU from Dr. Scherer, Cornell Medical College	
Ossabow		Vesicular stomatitis, New Jersey	
Alberta		EHD strain, prototype	
squirrel virus	squirrel blood	LaCrosse virus	
WiscAn5000-003	Whooping Crane	alphavirus	EEE virus
WiscAn5000-001	Whooping Crane	alphavirus	EEE virus
MF-8		VEE (1 AB) strain for early passage collection	
G8419		VEE (1 AB) strain for early passage collection	
T5-2092	Sciurus grisius	CTF-related	
S6-14-03	Lepus californicus	CTF-related	
2494, 2020, 2058 1833, 396, 848, 1602	mosquitoes	EEE strains from Massachusetts (1977-1984)	

Table 31 (continued)

Viruses referred to YARU for identification and study, 1983

Country of origin; strain	Source	Information from donor	YARU identification
Viet Nam			
Virus A	mosquitoes	dengue	not dengue
Virus B	mosquitoes	dengue	Japanese encephalitis
Virus C	mosquitoes	dengue	Japanese encephalitis
VN 58	pig blood	Japanese encephalitis	Japanese encephalitis
VN 78	human blood	dengue	Japanese encephalitis
VN 104	human blood	dengue	Japanese encephalitis
VN 113	human blood	dengue	Japanese encephalitis
VN 118	human blood	dengue	Japanese encephalitis
VN 131	human blood	dengue	not dengue
VN 135	human blood	dengue	Japanese encephalitis

END

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